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(54) Title: VIRUS ASSOCIATED MULTIPLE SCLEROSIS: TREATMENTS, PREVENTION AND DIAGNOSIS THEREOF

(57) Abstract

This invention relates to methods for preventing and treating virus associated multiple sclerosis. The invention also provides for the herpesvirus associated with multiple sclerosis, methods for detecting the virus, diagnosing viral associated multiple sclerosis, and methods for screening for herpesvirus associated multiple sclerosis.

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VIRUS ASSOCIATED MULTIPLE SCLEROSIS: TREATMENTS, PREVENTION AND DIAGNOSIS THEREOF

This application is a continuation-in-part of application Serial No. 08/287,942, filed on August 5, 1994, which is a continuation-in-part of application Serial No. 08/218,029, filed on March 24, 1994 (now abandoned), which is a continuation-in-part of application Serial No. 08/149,176, filed on November 5, 1993 (now abandoned), all of which are incorporated by reference herein.

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FIELD OF THE INVENTION

This invention relates to the discovery of the primary viral association for multiple sclerosis [MS]. The virus is a member of the Herpesvirus family and correlates statistically with patients suffering from MS. The invention also provides for methods of treating MS by conventional antiviral therapy and vaccines, for the predominant viral types associated with MS and for methods of diagnosing virally induced MS.

BACKGROUND OF THE INVENTION

This invention is the discovery of a virus associated type of multiple sclerosis (MS) and of methods for treating virally induced MS.

There is a great need to elucidate pathogenesis for MS and for methods of treatment such as are provided herein. MS is an inflammatory disease of the central nervous system. MS, with onset typically in the 3rd or 4th decade of life, is characterized clinically by a variable relapsing and remitting course and pathologically by the progressive accumulation of plaques of demyelination within the white matter of the brain, optic nerves, and spinal cord. Evidence suggests that the demyelination is mediated by macrophages and subsets of T-lymphocytes that are sensitized to components of myelin, which surrounds the axons of the neurons. The disease is pleomorphic in its presentation, but classic features include impaired vision, nystagmus, dysarthria, decreased perception of vibration and position sense, ataxia, intention tremor, weakness or paralysis of limbs, spasticity, and bladder problems.

Conventional diagnosis typically requires proof of at least two episodes of neurologic deficit and lesions at more than one site within the central nervous system. Although the diagnosis of MS is largely clinical, three laboratory tests are particularly useful as diagnostic adjuncts: 1) immunoglobulins are elevated in the CSF (cerebrospinal fluid) and have an oligoclonal banding pattern in 70-90% of patients which can vary both between patients or

between plaques; 2) magnetic resonance imaging (MRI) or nuclear magnetic resonance (NMR) of the brain and spinal cord reveal lesions in nearly every case of clinically defined MS, especially in periventricular regions; and 3) the visual evoked response is abnormal in about 75% of cases, with a decrease in the initial amplitude and prolongation of the latency of the major positive peak. Other ancillary tests which may be useful include a decreased suppressor or cytotoxic T cell activity and antibodies to myelin basic protein (MBP).

Although a number of theories about the etiology and pathogenesis of this disease exist, epidemiologic evidence suggests that the disease may be infectious in etiology (Kurtzke, J.F., 1993, *Clinical Microbiology Reviews* 6:382). Controversy exists in the field as to whether viruses are implicated in the disease. Antibodies have been found in significant titers in MS patients to Herpesviruses 1, 2 and 6, Epstein-Barr virus, Varicella-Zoster, Cytomegalovirus, retroviruses such as HIV-1 and HTLV-1, rubella, mumps, simian virus 5 and measles virus. With respect to identification of viral nucleic acid sequences, however, studies have only sporadically demonstrated the presence of viral genomes in peripheral blood leukocytes or brains from MS patients. The DNA of Herpes simplex virus Type I (HSV1) has been detected by PCR in only 1/77 plaques from patients with MS, and herpes simplex virus 2 (HSV-2) was not detected (Nicoll, JA *et al.*, 1992, *J. Neurolog. Sci.* 113(2):144). Human herpesvirus-6 (HHV-6) was reportedly found by PCR in only 1/31 patients' peripheral blood leukocytes by PCR, and in 1/24 normal controls (Sola, P. *et al.*, 1993 *J. Neurol. Neurosurg. and Psych.* 16(8):917). There is a need for a confirming clinical test for MS. The discovery of the association of the herpesvirus with MS as disclosed herein permits clinicians to provide for more definitive diagnoses for persons suspected of having virus associated MS.

The applicants do not wish to be bound by the following comments; but, herpesvirus is not thought to be the sole cause of MS. The virus is thought to trigger a complex

immunological response in the host that produces the symptoms associated with MS. The described herpesvirus may not be the sole cause of MS symptoms; but, the results provided herein clearly provide for the first time, statistical evidence that a substantial number of MS patients harbor the virus and that the titers can be higher in the MS patients than in the persons infected with the virus yet not having the disease.

Using a subtractive amplification method described herein, it was determined that HHV-6 or their particular subtypes are the primary causal agent of MS in humans. The family *Herpesviridae* includes over 100 double stranded DNA viruses, of which six have been well characterized in humans; HSV-1, HSV-2, human cytomegalovirus (CMV), Varicella Zoster virus (VZV), Epstein-Barr virus (EBV), and HHV-6. The members of this family of viruses have been classified into three subfamilies: the Alphaherpesvirinae (including HSV-1, HSV-2, and VZV), the Betaherpesvirinae (CMV, HHV-6), and the Gammaherpesvirinae (EBV). On the basis of biologic properties, HHV-6, a member of the Betaherpesvirinae, has been demonstrated to share 66% hybridization homology with CMV within a conserved region, but shares little homology with other herpesviruses.

HHV-6, a recently described member of the *Herpesviridae*, is the causative agent of exanthema subitem, a viral infection which is highly prevalent in children and can be associated with hepatitis, encephalitis, and post-infectious chronic fatigue. Biological differences and restriction endonuclease analysis of HHV-6 isolates have demonstrated that there are two distinct classes of HHV-6: those that are related to the Z29 strain isolated from a patient from Zaire (and associated with most cases of exanthema subitem), and those that are related to the U1102 strain, isolated from a patient from Uganda. HHV-6 has fairly broad tropism to cells including T and B lymphocytes, glial cells, and megakaryocytes and has been detected in the CSF of infected children with neurological symptoms (Ablashi *et al.*, 1989 *Dev. Virol. Stand.* 70:139). Buchwald *et al.* have also reported the detection of actively replicating HHV-6 in the

lymphocytes of 70% of 113 patients with a chronic fatigue syndrome characterized by an immunologically mediated inflammatory process of the central nervous system with areas of demyelination detected by NMR (Buchwald D., *et al.*, 1992, *Ann. Internal. Med.* 116(2):103).

Sporadic reports have also implicated HHV-6 in a number of diseases other than exanthema subitem and chronic fatigue syndrome, including Hodgkin's disease, inflammatory bowel disease, Sjogren's syndrome, sarcoidosis, retinitis, lymphoma, in conjunction with HTLV-I leukemia, and coinfecting a high percentage of patients with AIDS (Buchbinder *et al.*, 1988, *J. Virol. Meth.* 21 (1-4):191). Except for exanthema subitem, however, the exact role of HHV-6 in the pathogenesis of these diseases has not been established. A report that investigated the role of HHV-6 in MS described screening peripheral blood leukocytes which failed to show an association between the presence of the viral genome and the disease. Sola *et al.*, *supra*. In that study, the authors did not look for the presence of the virus in the central nervous system from these patients, where the primary lesions of MS occur.

Treatment for MS is largely ineffective and has focused on modulating the immune response with immunosuppressants or anti-inflammatory agents. Immunosuppressants such as glucocorticoids, adrenocorticotropic hormone (ACTH) to stimulate endogenous glucocorticoid production, azathioprine, cyclophosphamide or cyclosporine have been standard therapy. More recent attempts to modulate the immune response have included therapeutic regimens such as total lymphoid irradiation, passive or active immunotherapy, leukapheresis, using immune modulators such as mitoxantrone or levamisole, desensitization with MBP copolymer I, or treatment with interferons (IFN). Several clinical trials with interferons have demonstrated that interferon-γ increases the exacerbation rate of the disease, whereas both IFN-α and IFN-β have been found to inhibit disease activity (Panitch, H.S., 1992, *Drugs* 44(6):946; Knobler, R., 1988, *Neurology* 38 (7 Suppl. 2):58; Noronha, A., 1992, *Ann. Neurol.* 31(1):103). These responses

may be due to the augmenting effect that IFN-α and INF-β have on deficient suppressor activity in MS patients (Noronha, A., 1992, *Ann. Neurol.* 31(1):103 and Kastrukoff, 1991, *Neurology* 41(12):1936).

Thus, there remains a need for the conclusive identification of the causal agent for MS and for nucleic acid analysis techniques to detect such elusive pathogens. Once such agents are identified, definitive diagnostic assays are possible and treatments can be more appropriately designed.

SUMMARY OF THE INVENTION

HHV-6 is a member of the Herpesviridae that comprises two types: A and B, and within the B strains, types B1 and B2. We have determined that MS is caused by HHV-6 and particular substrains that include characteristics such as nucleic acid changes that are identifiable as genetic markers, quantitative differences in viral titer and localization to particular cell types within the brains of patients with MS. Where a substrain is determined to be neurotropic we designate it HHV-6^n.

This invention provides for methods for treatment of multiple sclerosis (MS) comprising administering to a patient having virus associated MS a pharmaceutically effective amount of an antiviral agent in a pharmaceutically acceptable carrier said agent wherein the agent is effective against HHV-6^n, with the proviso that the agent not be an interferon unless said interferon is in combination with another antiviral agent or applied directly to cerebrospinal fluids. A preferred antiviral agent is a nucleoside analogue such as those selected from the group consisting of acyclovir, cygalovir, and ganciclovir.

The invention also provides for the use of the anti-herpesvirus agents as identified herein for the manufacture of medicaments or therapuetics for the purpose of treating, preventing or alleviating the symptoms of multiple sclerosis. In addition, the medicaments themselves are also described herein. Especially preferred are those medicaments designed for intrathecal administration to the cerebrospinal fluid.

In addition to methods for treatment of MS, this invention provides for an isolated subtype of the *Herpesviridae* family wherein the virus has the following DNA subsequence in its genome:

MS: TATTCCCTATTAGCCAAGCTTACAAATTTCTCTAATGTCAA Seq. ID. No. 17.

This invention further provides for a method of screening for pharmaceutical agents useful in reducing the symptoms of multiple sclerosis (MS) comprising: contacting a cell infected with HHV-6 with an anti-viral agent; and, assaying the anti-viral agent activity by determining the effect of the agent upon viral titer in the cell. The method may be specifically designed to detect a member of the HHV-6^n subfamily of *Herpesviridae* especially those members having Seq. ID. No. 17. The methods can be designed to screen for agents in *in vitro* assays against cell lines infected with the virus, against cells producing an enzyme from a virus or against a purified viral enzyme. Alternatively, the agents may be screened in *in vivo assays* where the virus is hosted by a mammal.

This invention also provides for methods of diagnosising multiple sclerosis (MS) in a patient comprising the step of testing for the presence or absence of HHV-6 in a sample from a patient to be tested for MS. The virus to be detected can be any of the viruses described above. The method of detecting the virus can optionally involve nucleic acid hybridization methods which are selective and specific for the viruses described herein. The detection methods can also involve conventional immunoassays such as Western Blots, enzyme linked immunoassays (ELISA) and radioimmuno assays (RIA). Preferred tissues samples include nerve tissue such as brain samples. The diagnosis for MS can include conventional criteria or diagnostic factors in combination with those disclosed herein. The impact of treatment upon the disease progression can be assessed using these methods at different times.

Having determined that a virus is responsible for MS, there is also disclosed herein a method of prophylaxis for

multiple sclerosis (MS) by administering to a patient at risk for MS, a vaccine against MS comprising inactivated viral particles or peptides of a virus from HHV-6 in a pharmaceutically acceptable carrier wherein the vaccine is effective against HHV-6 wherein the virus is as described herein and is typified by MSV-1206.

The invention further provides for DNA probes and PCR primers that are specific to the *Herpesviridae* family of virus in that they can, under appropriately stringent hybridization conditions, bind selectively and specifically to the virus in the presence of a human genome. Table 1 provides a list of preferred primers, regions for probe to bind and probes that can distinguish between MS associated HHV-6 and non-MS associated HHV-6.

This invention also provides for diagnostic kits for the diagnosis of multiple sclerosis (MS) comprising a container having an antibody or a nucleic acid probe that is specific for the HHV-6^n identified herein as associated with MS such as MSV-1206.

DESCRIPTION OF THE FIGURES

Figure 1 is a map of the HHV-viral genome. PZVH14 contains a *Hind*III site which distinguishes HHV-6 Types A and B. The polymerase region is designated "pol". The glycoprotein B region is designated "gB". The DNA binding protein region is designated "DBP" and contains a *Hind*III site that is diagnostic for virus MSV-1206. The variable glycoprotein region is designated "vg". The glycoprotein H region is designated "gH" and allows the discrimination of HHV-6 Type B group 1 and group 2 viruses. The immediate early gene region is designated "IE" and also allows the discrimination of Type B group 1 and group 2 viruses.

Figure 2 is a map of marker sites within the HHV-6 genome that distinguish between Group B2 viruses. These markers are used to distinguish and characterize the MS virus from those found in controls.

DETAILED DESCRIPTION

We have identified a viral agent consistently associated in MS. With this information, it is now possible to diagnose the disease more accurately and earlier, to treat the disease, to prevent the disease, and to screen for and develop new treatments and prophylactics for the disease.

I. Treatment of viral induced MS.

With the discovery of the disease causal agent for MS, now identified, effective therapeutic protocols to alleviate the symptoms of HHV-6 associated MS can be formulated. Due to the viral nature of the disease, antiviral agents have application here for treatment, such as interferons, nucleoside analogues, ribavirin, amantadine, and pyrophosphate analogues of phosphonoacetic acid (foscarnet) (reviewed in Gorbach, S.L., *et al.*, 1992, *Infectious Diseases* Ch.35:289, W.B. Saunders, Philadelphia, PA) and the like. Immunological therapy will also be effective in many cases to manage and alleviate symptoms caused by the disease agents described here. Antiviral agents include agents or compositions that directly bind to viral products and interfere with disease progress; and, excludes agents that do not impact directly on viral multiplication or viral titer. Antiviral agents do not include immunoregulatory agents that do not directly affect viral titer or bind to viral products. Antiviral agents are effective if they inactivate the virus, otherwise inhibit its infectivity or multiplication, or alleviate the symptoms of MS.

A. Antiviral Agents.

i) Introduction

The antiherpesvirus agents that will be useful for treating virus-induced MS can be grouped into broad classes based on their presumed modes of action. These classes include agents that act (i) by inhibition of viral DNA polymerase, (ii) by targeting other viral enzymes and proteins, (iii) by miscellaneous or incompletely understood mechanisms, or (iv) by binding a target nucleic acid (*i.e.*, inhibitory nucleic acid therapeutics). Antiviral agents may also be used in combination (*i.e.*, together or sequentially)

to achieve synergistic or additive effects or other benefits. Several compositions have been reported to modulate the immune system and affect the progression of MS. These compounds include β-interferon and its analog Betaseron. In addition, the immunosupressant drug cladribine, a lympholytic agent, was also reported to be of use for treating MS. All of these drugs are applied intravenously and are not applied directly to cerebrospinal tissue or fluids. In contrast, this invention specifically suggests that the antiviral effects of interferons (α or β) and the other compositions would be enhanced if applied directly to such tissue or fluids for patients suffering from HHV-6 infections leading to MS. To the extent that drugs such as β-interferon, Betaseron and cladribine have antiviral effects, the direct administration or application of these agents to the cerbrospinal fluids or tissues for treating HHV infections is a part of this invention.

Although it is convenient to group antiviral agents by their supposed mechanism of action, the applicants do not intend to be bound by any particular mechanism of antiviral action. Moreover, it will be understood by those of skill that an agent may act on more than one target in a virus or virus-infected cell or through more than one mechanism.

ii) Inhibitors of viral DNA polymerase

Many antiherpesvirus agents in clinical use or in development today are nucleoside analogs believed to act through inhibition of viral DNA replication, especially through inhibition of viral DNA polymerase. These nucleoside analogs act as alternative substrates for the viral DNA polymerase or as competitive inhibitors of DNA polymerase substrates. Usually these agents are preferentially phosphorylated by viral thymidine kinase (TK), if one is present, and/or have higher affinity for viral DNA polymerase than for the cellular DNA polymerases, resulting in selective antiviral activity. Where a nucleoside analogue is incorporated into the viral DNA, viral activity or reproduction may be affected in a variety of ways. For example, the analogue may act as a chain terminator, cause

increased lability (*e.g.*, susceptibility to breakage) of analogue-containing DNA, and/or impair the ability of the substituted DNA to act as template for transcription or replication (*see, e.g.*, Balzarini *et al.* (1990) *Mol. Pharm.* 37, 402-7).

It will be known to one of skill that, like many drugs, many of the agents useful for treatment of herpes virus infections are modified (*i.e.*, "activated") by the host, host cell, or virus-infected host cell metabolic enzymes. For example, acyclovir is triphosphorylated to its active form, with the first phosphorylation being carried out by the herpes virus thymidine kinase, when present. Other examples are the reported conversion of the compound HOE 602 to ganciclovir in a three-step metabolic pathway (Winkler *et al.* (1990) *Antiviral Research* 14: 61-74) and the phosphorylation of ganciclovir to its active form by, *e.g.*, a CMV nucleotide kinase. It will be apparent to one of skill that the specific metabolic capabilities of a virus can affect the sensitivity of that virus to specific drugs, and is one factor in the choice of an antiviral drug. For example, ganciclovir is preferred over acyclovir for treatment of CMV infection because CMV does not express a viral thymidine kinase that phosphorylates acyclovir. In the case of HHV-6, early reports suggest that HHV-6 is more sensitive to ganciclovir than to acyclovir, although acyclovir does inhibit HHV-6 viral DNA replication (Di Luca et al. (1990) *Virology* 175(1): 199-210; Burns and Sandford, *J. Infect. Dis.* (1990) 162(3):634-7). The mechanism of action of certain anti-herpesvirus agents is discussed in De Clercq, *Antimicrobial Chemotherapy* (1993) 32, Suppl. A, 121-132 and in other references cited *supra* and *infra*, all of which are incorporated by reference herein.

Anti-herpesvirus medications suitable for treating viral induced MS include, but are not limited to, nucleoside analogs including acyclic nucleoside phosphonate analogs (*e.g.*, phosphonylmethoxyalkylpurines and -pyrimidines), and cyclic nucleoside analogs. These include drugs such as: vidarabine (9-β-D-arabinofuranosyladenine; adenine arabinoside, ara-A, Vira-A, Parke-Davis); 1-β-D-

arabinofuranosyluracil (ara-U); 1-β-D-arabinofuranosyl-cytosine (ara-C); HPMPC [(S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (*e.g.*, GS 504 Gilead Science)] and its cyclic form (cHPMPC); HPMPA [(S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] and its cyclic form (cHPMPA); (S)-HPMPDAP [(S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)-2,6-diaminopurine]; PMEDAP [9-(2-phosphonyl-methoxyethyl)-2,6-diaminopurine]; HOE 602 [2-amino-9-(1,3-bis(isopropoxy)-2-propoxymethyl)purine]; PMEA [9-(2-phosphonylmethoxyethyl)adenine]; bromovinyl-deoxyuridine (Burns and Sandford., J. Infect. Dis. (1990) 162(3):634-7); 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)-uridine or -2'-deoxyuridine; BVaraU (1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)-uracil, brovavir, Bristol-Myers Squibb, Yamsa Shoyu); BVDU [(E)-5-(2-bromovinyl)-2'-deoxyuridine, brivudin, *e.g.*, Helpin] and its carbocyclic analogue (in which the sugar moiety is replaced by a cyclopentane ring); IVDU [(E)-5-(2-iodovinyl)-2'-deoxyuridine] and its carbocyclic analogue, C-IVDU (Balzarini *et al.*, 1990, *Mol. Pharm.*, 37, 402-407)]; and 5-mercutithio analogs of 2'-deoxyuridine (Holliday, J., and Williams, M.V., 1992, *Antimicrob. Agents Chemother.* 36(9):1935); acyclovir [9-([2-hydroxyethoxy]methyl)guanine; *e.g.*, Zovirax (Burroughs Wellcome)]; penciclovir (9-[4-hydroxy-2-(hydroxymethyl)butyl]-guanine); ganciclovir [(9-[1,3-dihydroxy-2 propoxymethyl]-guanine) *e.g.*, Cymevene, Cytovene (Syntex), DHPG (Stals *et al.* (1993) *Antimicrobial Agents Chemother.* 37(2):218-23]; isopropylether derivatives of ganciclovir (*see, e.g.*, Winkelmann *et al.*, 1988, *Drug Res.* 38, 1545-48); cygalovir; famciclovir [2-amino-9-(4-acetoxy-3-(acetoxymethyl)but-1-yl)purine (Smithkline Beecham)]; valacyclovir (Burroughs Wellcome); desciclovir [(2-amino-9-(2-ethoxymethyl)purine)] and 2-amino-9-(2-hydroxyethoxymethyl)-9H-purine, prodrugs of acyclovir]; CDG (carbocyclic 2'-deoxyguanosine); and purine nucleosides with the pentafuranosyl ring replaced by a cyclo butane ring (*e.g.*, cyclobut-A [(+-)-9-[1β,2α,3β)-2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine], cyclobut-G [(+-)-9-[1β,2α,3β)-2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine], BHCG [(R)-

(1α,2β,1α)-9-(2,3-bis(hydroxymethyl)cyclobutyl]guanine], and an active isomer of racemic BHCG, SQ 34,514 [1R-1α,2β,3α)-2-amino-9-[2,3-bis(hydroxymethyl)cyclobutyl]-6H-purin-6-one (*see*, Braitman et al.(1991) *Antimicrob. Agents and Chemotherapy* 35(7):1464-8)]. Certain of these antiherpesviral agents are discussed in Gorach *et al.*, 1992, *Infectious Diseases*, Chapter 35; Saunders *et al.*, 1990, *Antiviral Res.* 13 (1):41; Yamanaka *et al.*, 1991. *Mol. Pharmacol.* 40(3):446 (1991); Greenspan *et al.*, 1990, *J. Acquir. Immune Defic. Syndr.* 3 (6):571), all of which are incorporated by reference herein.

Triciribine and triciribine monophosphate are potent inhibitors against HHV-6 (Ickes *et al.*, Seventh International Conf. on Antiviral Research 1994, Abstract No. 122 in *Antiviral Research* 23 (Supp. 1) (1994), incorporated by reference herein), HIV-1 and HIV-2 (Kucera *et al.*, 1993, *AIDS Res. Human Retroviruses* 9:307-314, incorporated by reference herein) and are additional nucleoside analogs that may be used to treat MS. An exemplary protocol for these agents is an intravenous injection of about 0.35 mg/meter^2 (0.7 mg/kg) once weekly or every other week for at least two doses, preferably up to about four to eight weeks.

Acyclovir and ganciclovir are of particular interest and are preferred because of their accepted use in clinical settings. Acyclovir, an acyclic analogue of guanine, is phosphorylated by a herpesvirus thymidine kinase and undergoes further phosphorylation to be incorporated as a chain terminator by the viral DNA polymerase during viral replication. It has therapeutic activity against a broad range of herpesviruses, including HHV-6, Herpes simplex Types 1 and 2, Varicella- Zoster, Cytomegalovirus, and Epstein-Barr Virus, and is used to treat disease such as herpes encephalitis, neonatal herpesvirus infections, chickenpox in immunocompromised hosts, herpes zoster recurrences, CMV retinitis, EBV infections, chronic fatigue syndrome, and hairy leukoplakia in AIDS patients. Exemplary intravenous dosages or oral dosages for MS are 250 mg/kg/m^2 body surface area, every 8 hours for 7 days, or maintenance doses of 200-400 mg

IV or orally twice a day to suppress recurrence. Ganciclovir has been shown to be more active than acyclovir against herpesviruses, including HHV-6 and CMV. *See, e.g.*, Oren and Soble, 1991, *Clinical Infectious Diseases* 14:741-6. Treatment protocols for ganciclovir are 5 mg/kg twice a day IV or 2.5 mg/kg three times a day for 10-14 days. Maintenance doses are 5-6 mg/kg for 5-7 days.

Also of particular interest is HPMPC. HPMPC is reported to be more active than either acyclovir or ganciclovir in the chemotherapy and prophylaxis of various HSV-1, HSV-2, TK- HSV, VZV or CMV infections in animal models (De Clercq, *supra*). Compounds with anti-CMV activity are likely to be particularly useful in treatment of viral induced MS because, apart from HHV-7, CMV appears to be the herpesvirus most closely related to HHV-6.

Nucleoside analogs such as BVaraU are potent inhibitors of HSV-1, EBV, and VZV that have greater activity than acyclovir in animal models of encephalitis. FIAC (fluroidoarbinosyl cytosine) and its related fluroethyl and iodo compounds (*e.g.*, FEAU, FIAU) have potent selective activity against herpesviruses, and HPMPA ((S)-1-([3-hydroxy-2-phosphorylmethoxy]propyl)adenine) has been demonstrated to be more potent against HSV and CMV than acyclovir or ganciclovir and are of choice in advanced cases of MS. Cladribine (2-chlorodeoxyadenosine) is another nucleoside analogue known as a highly specific antilymphocyte agent (*i.e.*, a immunosuppressive drug). Cladribine has been found useful in treatment of chronic progressive MS (Sipe *et al.*, (1994) *Lancet* 344:9-13).

Other useful antiviral agents include: 5-thien-2-yl-2'-deoxyuridine derivatives, *e.g.*, BTDU [5-5(5-bromothien-2-yl)-2'-deoxyuridine] and CTDU [b-(5-chlorothien-2-yl)-2'-deoxyuridine]; and OXT-A [9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanosyl)adenine] and OXT-G [9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanosyl)guanine]. Although OXT-G is believed to act by inhibiting viral DNA synthesis its mechanism of action has not yet been elucidated. These and other compounds are described in Andrei *et al.* (1992) *Eur. J.*

Clin. Microbiol. Infect. Dis. 11(2):143-51 which is incorporated by reference herein. Additional antiviral purine derivatives useful in treating herpesvirus infections are disclosed in US Pat. 5,108,994 (assigned to Beecham Group P.L.C.). 6-Methoxypurine arabinoside (ara-M; Burroughs Wellcome) is a potent inhibitor of varicella-zoster virus, and will be useful for treatment of herpesvirus-induced MS.

Certain thymidine analogs [*e.g.*, idoxuridine (5-ido-2'-deoxyuridine)] and triflurothymidine) have antiherpes viral activity, but due to their systemic toxicity, are largely used for topical herpesviral infections, including HSV stromal keratitis and uveitis, and are not preferred here unless other options are ruled out.

Bodor (U.S. Patent No. 5,177,064) has described compounds wherein one moiety is the residue of a drug, such as an antiherpesvirus agent, and the second moiety is a phosphonate ester. These phosphonate derivatives are adapted for targeted delivery, especially to the brain and will be useful in treatment of virus-induced MS.

Other useful antiviral agents that have demonstrated antiherpes viral activity include foscarnet sodium (trisodium phosphonoformate, PFA, Foscavir (Astra)) and phosphonoacetic acid (PAA). Foscarnet is an inorganic pyrophosphate analogue that acts by competitively blocking the pyrophosphate-binding site of DNA polymerase. These agents which block DNA polymerase directly without processing by viral thymidine kinase. Foscarnet is reported to be less toxic than PAA.

iii) Agents that target viral proteins other than DNA polymerase or other viral functions.

Although applicants do not intend to be bound by a particular mechanism of antiviral action, the antiherpes-virus agents described above are believed to act through inhibition of viral DNA polymerase. However, viral replication requires not only the replication of the viral nucleic acid but also the production of viral proteins and other essential components. Accordingly, the present invention contemplates treatment of MS by the inhibition of viral proliferation by targeting viral proteins other than DNA polymerase (*e.g.*, by

inhibition of their synthesis or activity, or destruction of viral proteins after their synthesis). For example, administration of agents that inhibit a viral serine protease, *e.g.*, such as one important in development of the viral capsid will be useful in treatment of viral induced MS. An example of such a serine protease is the HHV-6 homolog of the HSV U_L26 gene product described in EP 0 514 830 A2, which is herein incorporated by reference.

Other viral enzyme targets include: OMP decarboxylase inhibitors (a target of, *e.g.*, parazofurin), CTP synthetase inhibitors (targets of, e.g., cyclopentenylcytosine), IMP dehydrogenase, ribonucleotide reductase (a target of, *e.g.*, carboxyl-containing N-alkyldipeptides as described in U.S. Patent No. 5,110,799 (Tolman *et al.*, Merck)), thymidine kinase (a target of, *e.g.*, 1-[2-(hydroxymethyl)cylcoalkylmethyl]-5-substituted -uracils and -guanines as described in, *e.g.*, U.S. Patent Nos. 4,863,927 and 4,782,062 (Tolman *et al.*; Merck)) as well as other enzymes. One target is an HHV-6 phosphotransferase that is a homolog of the HSV U_L13 gene product described in PCT Application WO 94/04920, which is incorporated by reference herein. It will be apparent to one of ordinary skill in the art that there are additional viral proteins, both characterized and as yet to be discovered, that can serve as target for antiviral agents.

iv) Other agents and modes of antiviral action.

In addition to the above antiviral agents, Kutapressin has been recently reported as having efficacy against HHV-6 virus infections. Kutapressin is a liver derivative available from Schwarz Parma of Milwaukee, Wisconsin in an injectable form of 25 mg/ml. The recommended dosage for HHV-6 is from 200 to 25 mg/ml per day for an average adult of 150 pounds.

Poly(I)·Poly(C_{12}U), an accepted antiviral drug known as Ampligen from HEM Pharmaceuticals of Rockville, MD has been shown to inhibit HHV-6 and is another antiviral agent suitable for treating MS. Intravenous injection is the preferred route of administration. Dosages from about 100 to 600 mg/m^2 are

administered two to three times weekly to adults averaging 150 pounds. It is best to administer at least 200 mg/m^2 per week.

Other antiviral agents reported to show activity against herpes viruses (*e.g.*, varicella zoster and herpes simplex) and will be useful for the treatment of viral induced MS include mappicine ketone (SmithKline Beecham); Compounds A,79296 and A,73209 (Abbott) for varicella zoster, and Compound 882C87 (Burroughs Wellcome) [*see, The Pink Sheet* 55(20) May 17, 1993].

Interferon is known inhibit HHV-6 replication. *See,* Oren and Sobel, 1991, *supra*. Interferon has known toxicity problems and it is expected that second generation derivatives will soon be available that will retain interferon's antiviral properties but have reduced side affects.

It is also contemplated that viral induced MS may be treated by administering a HHV-6 reactivating agent to induce reactivation of the latent virus. Preferably the reactivation is combined with simultaneous or sequential administration of an anti-HHV-6 agent. Controlled reactivation over a short period of time or reactivation in the presence of an antiviral agent is believed to minimize the adverse effects of certain herpesvirus infections (*e.g.*, as discussed in PCT Application WO 93/04683). Reactivating agents include agents such as estrogen, phorbol esters, forskolin and β-adrenergic blocking agents.

Agents useful for treatment of herpesvirus infections and for treatment of virus-induced MS are described in numerous U.S. Patents. For example, ganciclovir is an example of a antiviral guanine acyclic nucleoside of the type described in US Patent Nos. 4,355,032 and 4,603,219.

Acyclovir is an example of a class of antiviral purine derivatives, including 9-(2-hydroxyethylmethyl)adenine, of the type described in U.S. Pat. Nos. 4,287,188, 4,294,831 and 4,199,574.

Brivudin is an example of an antiviral deoxyuridine derivative of the type described in US Patent No. 4,424,211.

Vidarabine is an example of an antiviral purine nucleoside of the type described in British Pat. 1,159,290.

Brovavir is an example of an antiviral deoxyuridine derivative of the type described in US Patent Nos. 4,542,210 and 4,386,076.

BHCG is an example of an antiviral carbocyclic nucleoside analogue of the type described in US Patent Nos. 5,153,352, 5,034,394 and 5,126,345.

HPMPC is an example of an antiviral phosphonyl methoxyalkyl derivative with of the type described in US Patent No. 5,142,051.

CDG (Carbocyclic 2'-deoxyguanosine) is an example of an antiviral carbocyclic nucleoside analogue of the type described in US Patent Nos. 4,543,255, 4,855,466, and 4,894,458.

Foscarnet is described in US Patent No. 4,339,445.

Trifluridine and its corresponding ribonucleoside is described in US Patent No. 3,201,387.

U.S. Patent No. 5,321,030 (Kaddurah-Daouk *et al.*; Amira) describes the use of creatine analogs as antiherpes viral agents. U.S. Patent No. 5,306,722 (Kim *et al.*; Bristol-Meyers Squibb) describes thymidine kinase inhibitors useful for treating HSV infections and for inhibiting herpes thymidine kinase. Other anitherpesvirus compositions are described in U.S. Patent Nos. 5,286,649 and 5,098,708 (Konishi *et al.*, Bristol-Meyers Squibb) and 5,175,165 (Blumenkopf *et al.*; Burroughs Wellcome). U.S. Patent No. 4,880,820 (Ashton *et al.*; Merck) describes the antiherpes virus agent (S)-9-(2,3-dihydroxy-1-propoxymethyl)guanine.

U.S. Patent No. 4,708,935 (Suhadolnik *et al.*; Research Corporation) describes a 3'-deoxyadenosine compound effective in inhibiting HSV and EBV. U.S. Patent No. 4,386,076 (Machida *et al.*; Yamasa Shoyu Kabushiki Kaisha) describes use of (E)-5-(2-halogenovinyl)-arabinofuranosyluracil as an antiherpesvirus agent. U.S. Patent No. 4,340,599 (Lieb *et al.*; Bayer Aktiengesellschaft) describes phosphonohydroxyacetic acid derivatives useful as antiherpes agents. U.S. Patent Nos. 4,093,715 and 4,093,716 (Lin *et al.* Research Corporation) describe 5'-amino-5'-deoxythymidine and

5-iodo-5'-amino-2',5'-dideoxycytidine as potent inhibitors of herpes simplex virus. U.S. Patent No. 4,069,382 (Baker *et al.*; Parke, Davis & Company) describes 9-(5-O-Acyl-beta-D-arabinofuranosyl)adenine compounds useful as antiviral agents. U.S. Patent No. 3,927,216 (Witkowski *et al.*) describes the use of 1,2,4-triazole-3-carboxamide and 1,2,4-triazole-3-thiocarboxamide for inhibiting herpes virus infections. Patent No. 5,179,093 (Afonso *et al.*, Schering) describes quinoline-2,4-dione derivatives active against herpes simplex virus 1 and 2, cytomegalovirus and Epstein Barr virus.

v) Inhibitory nucleic acid therapeutics

Also contemplated here are inhibitory nucleic acid therapeutics which can inhibit the activity of HHV-6 viruses in patients with MS. Inhibitory nucleic acids may be single-stranded nucleic acids, which can specifically bind to a complementary nucleic acid sequence. By binding to the appropriate target sequence, an RNA-RNA, a DNA-DNA, or RNA-DNA duplex or triplex is formed. These nucleic acids are often termed "antisense" because they are usually complementary to the sense or coding strand of the gene, although recently approaches for use of "sense" nucleic acids have also been developed. The term "inhibitory nucleic acids" as used herein, refers to both "sense" and "antisense" nucleic acids.

By binding to the target nucleic acid, the inhibitory nucleic acid can inhibit the function of the target nucleic acid. This could, for example, be a result of blocking DNA transcription, processing or poly(A) addition to mRNA, DNA replication, translation, or promoting inhibitory mechanisms of the cells, such as promoting RNA degradation. Inhibitory nucleic acid methods therefore encompass a number of different approaches to altering expression of, for example, HHV-6^n genes that operate by different mechanisms. These different types of inhibitory nucleic acid technology are described in Helene, C. and Toulme, J., 1990, *Biochim. Biophys. Acta.* 1049:99-125, which is hereby incorporated by reference and is referred to hereinafter as "Helene and Toulme."

In brief, inhibitory nucleic acid therapy approaches can be classified into those that target DNA sequences, those that target RNA sequences (including pre-mRNA and mRNA), those that target proteins (sense strand approaches), and those that cause cleavage or chemical modification of the target nucleic acids.

Approaches targeting DNA fall into several categories. Nucleic acids can be designed to bind to the major groove of the duplex DNA to form a triple helical or "triplex" structure. Alternatively, inhibitory nucleic acids are designed to bind to regions of single stranded DNA resulting from the opening of the duplex DNA during replication or transcription. *See* Helene and Toulme.

More commonly, inhibitory nucleic acids are designed to bind to mRNA or mRNA precursors. Inhibitory nucleic acids are used to prevent maturation of pre-mRNA. Inhibitory nucleic acids may be designed to interfere with RNA processing, splicing or translation.

The inhibitory nucleic acids can be targeted to mRNA. In this approach, the inhibitory nucleic acids are designed to specifically block translation of the encoded protein. Using this approach, the inhibitory nucleic acid can be used to selectively suppress certain cellular functions by inhibition of translation of mRNA encoding critical proteins. For example, an inhibitory nucleic acid complementary to regions of c-myc mRNA inhibits c-myc protein expression in a human promyelocytic leukemia cell line, HL60, which overexpresses the c-myc proto-oncogene. *See* Wickstrom E.L., *et al.*, 1988, *PNAS (USA)* 85:1028-1032 and Harel-Bellan, A., *et al.*, 1988, *Exp. Med.* 168:2309-2318. As described in Helene and Toulme, inhibitory nucleic acids targeting mRNA have been shown to work by several different mechanisms to inhibit translation of the encoded protein(s).

The inhibitory nucleic acids introduced into the cell can also encompass the "sense" strand of the gene or mRNA to trap or compete for the enzymes or binding proteins involved in mRNA translation. *See* Helene and Toulme.

Lastly, the inhibitory nucleic acids can be used to induce chemical inactivation or cleavage of the target genes or mRNA. Chemical inactivation can occur by the induction of crosslinks between the inhibitory nucleic acid and the target nucleic acid within the cell. Other chemical modifications of the target nucleic acids induced by appropriately derivatized inhibitory nucleic acids may also be used.

Cleavage, and therefore inactivation, of the target nucleic acids may be effected by attaching a substituent to the inhibitory nucleic acid which can be activated to induce cleavage reactions. The substituent can be one that affects either chemical, or enzymatic cleavage. Alternatively, cleavage can be induced by the use of ribozymes or catalytic RNA. In this approach, the inhibitory nucleic acids would comprise either naturally occurring RNA (ribozymes) or synthetic nucleic acids with catalytic activity.

The targeting of inhibitory nucleic acids to specific cells of the immune system by conjugation with targeting moieties binding receptors on the surface of these cells can be used for all of the above forms of inhibitory nucleic acid therapy. This invention encompasses all of the forms of inhibitory nucleic acid therapy as described above and as described in Helene and Toulme.

This invention relates to the targeting of inhibitory nucleic acids to sequences of HHV-6 for use in treating MS. An example of an antiherpes virus inhibitory nucleic acid is ISIS 2922 (ISIS Pharmaceuticals) which has activity against CMV [*see, Biotechnology News* 14(14) p. 5].

A problem associated with inhibitory nucleic acid therapy is the effective delivery of the inhibitory nucleic acid to the target cell *in vivo* and the subsequent internalization of the inhibitory nucleic acid by that cell. This can be accomplished by linking the inhibitory nucleic acid to a targeting moiety to form a conjugate that binds to a specific receptor on the surface of the target infected cell, and which is internalized after binding.

vi) Combinations

The use of combinations of antiviral drugs and sequential treatments are useful for treatment of herpesvirus infections and will also be useful for the treatment of viral induced MS. For example, Snoeck *et al.* (1992) *Eur. J. Clin. Micro. Infect. Dis.* 11(12) 1144-55, found additive or synergistic effects against CMV when combining antiherpes drugs (*e.g.*, combinations of zidovudine [3'-azido-3'-deoxythymidine, AZT] with HPMPC, ganciclovir, foscarnet or acyclovir or of HPMPC with other antivirals). Similarly, in treatment of cytomegalovirus retinitis, induction with ganciclovir followed by maintenance with foscarnet has been suggested as a way to maximize efficacy while minimizing the adverse side effects of either treatment alone. An anti-herpetic composition that contains acyclovir and, *e.g.*, 2-acetylpyridine-5-((2-pyridylamino)thiocarbonyl)-thiocarbonohydrazone is described in U.S. Pat. 5,175,165 (assigned to Burroughs Wellcome Co.). Combinations of TS-inhibitors and viral TK-inhibitors in antiherpetic medicines are disclosed in U.S. Pat. 5,137,724, assigned to Stichting Rega VZW. A synergistic inhibitory effect on EBV replication using certain ratios of combinations of HPMPC with AZT was reported by Lin *et al.* (1991) *Antimicrob Agents Chemother* 35(11): 2440-3. Acyclic nucleoside analogs have also been shown to have synergistic antiherpesvirus activity when combined with interferon or its less toxic second generation derivatives and are contemplated here for MS (Taylor, J.L., 1991, *Curr. Eye Res.*, 10 Suppl.:205; O'Brien, W.J., *et al.*, 1990, *Antimicrob. Agents Chemother.* 34(6):1178). The use of antiherpes agents (*e.g.*, trifluridine) in combination with immunosuppressive agents (*e.g.*, prednisolone) has also provided benefit for certain patients. See, O'Brien and Taylor (1991) Invest. Ophthalmol. Vis. Sci. 32(9):2455-61. Combinations of drugs may reduce toxicity, *e.g.*, because lower doses of individual agents may be required for efficacy.

U.S. Patent Nos. 5,164,395 and 5,021,437 (Blumenkopf; Burroughs Wellcome) describe the use of a ribonucleotide reductase inhibitor (an acetylpyridine

derivative) for treatment of herpes infections, including the use of the acetylpyridine derivative in combination with acyclovir. U.S. Patent No. 5,137,724 (Balzari *et al.*) describes the use of thymilydate synthase inhibitors (*e.g.*, 5-fluoro-uracil and 5-fluro-2'-deoxyuridine) in combination with compounds having viral thymidine kinase inhibiting activity.

vii) Administration

The antiviral compositions for treating MS are preferably administered to human patients via oral, intravenous or parenteral administrations and other systemic forms. Intrathecal administration is also contemplated or are administered as discussed above in connection with the individual compositions. Those of skill in the art will understand appropriate administration protocol for the individual compositions to be employed by the physician. A preferred route is via a permanent intrathecal catheter for administration (both intermittent and continuous) of antiviral agents.

The pharmaceutical formulations or compositions of this invention may be in the dosage form of solid, semi-solid, or liquid such as, *e.g.*, suspensions, aerosols or the like. Preferably the compositions are administered in unit dosage forms suitable for single administration of precise dosage amounts. The compositions may also include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological saline, Ringer's solution, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants; or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like. Effective amounts of such diluent or carrier are those amounts which are effective to obtain a pharmaceutically acceptable formulation in terms of solubility of components, or biological activity, etc.

All patents, publications, and patent applications cited herein are incorporated herein by reference. In addition, applicants expressly disclaim any agent that has been previously tested successfully in MS patients due the agent's putative immunosuppressive properties but which in reality was effective due to the agent's unappreciated antiviral properties, e.g. Betaseron.

B. Immunological Approaches to Therapy.

Having identified a primary causal agent of MS in humans as a virus, there are routine immunosuppressive therapies that can modulate the immunologic dysfunction that arises from the presence of viral infected nerve tissue. In particular, agents that block the immunological attack of the viral infected brain cells will ameliorate the neurological symptoms of MS and/or reduce the disease progress.

Such therapies include antibodies that specifically block the targeting of viral infected cells. Such agents include antibodies which bind to cytokines that upregulate the immune system to target viral infected cells.

The antibody may be administered to a patient either singly or in a cocktail containing two or more antibodies, other therapeutic agents, compositions, or the like, including, but not limited to, immunosuppressive agents, potentiators and side-effect relieving agents. Of particular interest are immunosuppressive agents useful in suppressing allergic reactions of a host. Immunosuppressive agents of interest include prednisone, prednisolone, DECADRON (Merck, Sharp & Dohme, West Point, PA), cyclophosphamide, cyclosporine, 6-mercaptopurine, methotrexate, azathioprine and i.v. gamma globulin or their combination. Potentiators of interest include monensin, ammonium chloride and chloroquine. All of these agents are administered in generally accepted efficacious dose ranges such as those disclosed in the *Physician Desk Reference*, 41st Ed. (1987), Publisher Edward R. Barnhart, New Jersey.

Immune globulin from persons previously infected with HHV-6 or related viruses can be obtained using standard techniques. Appropriate titers of antibodies are known for

this therapy and are readily applied to the treatment of MS. Immune globulin can be administered via parenteral injection or by intrathecal shunt. In brief, immune globulin preparations may be obtained from individual donors who are screened for antibodies to HHV-6, and plasmas from high-titered donors are pooled. Alternatively, plasmas from donors are pooled and then tested for antibodies to HHV-6; high-titered pools are then selected for use in MS patients.

Antibodies may be formulated into an injectable preparation. Parenteral formulations are known and are suitable for use in the invention, preferably for i.m. or i.v. administration. The formulations containing therapeutically effective amounts of antibodies or immunotoxins are either sterile liquid solutions, liquid suspensions or lyophilized versions and optionally contain stabilizers or excipients. Lyophilized compositions are reconstituted with suitable diluents, *e.g.*, water for injection, saline, 0.3% glycine and the like, at a level of about from .01 mg/kg of host body weight to 10 mg/kg where appropriate. Typically, the pharmaceutical compositions containing the antibodies or immunotoxins will be administered in a therapeutically effective dose in a range of from about .01 mg/kg to about 5 mg/kg of the treated mammal. A preferred therapeutically effective dose of the pharmaceutical composition containing antibody or immunotoxin will be in a range of from about 0.01 mg/kg to about 0.5 mg/kg body weight of the treated mammal administered over several days to two weeks by daily intravenous infusion, each given over a one hour period, in a sequential patient dose-escalation regimen.

Antibody may be administered systemically by injection i.m., subcutaneously, intrathecally or intraperitoneally or into vascular spaces, particularly into the joints, *e.g.*, intraarticular injection at a dosage of greater than about 1 μg/cc joint fluid/day. A permanent intrathecal catheter would be a convenient means to administer therapeutic antibodies. The dose will be dependent upon the properties of the antibody or immunotoxin employed, *e.g.*, its activity and biological half-life, the concentration of

antibody in the formulation, the site and rate of dosage, the clinical tolerance of the patient involved, the disease afflicting the patient and the like as is well within the skill of the physician.

The antibody of the present invention may be administered in solution. The pH of the solution should be in the range of pH 5 to 9.5, preferably pH 6.5 to 7.5. The antibody or derivatives thereof should be in a solution having a suitable pharmaceutically acceptable buffer such as phosphate, tris (hydroxymethyl) aminomethane-HCl or citrate and the like. Buffer concentrations should be in the range of 1 to 100 mM. The solution of antibody may also contain a salt, such as sodium chloride or potassium chloride in a concentration of 50 to 150 mM. An effective amount of a stabilizing agent such as an albumin, a globulin, a gelatin, a protamine or a salt of protamine may also be included and may be added to a solution containing antibody or immunotoxin or to the composition from which the solution is prepared.

Systemic administration of antibody is made daily, generally by intramuscular injection, although intravascular infusion is acceptable. Administration may also be intranasal or by other nonparenteral routes. Antibody or immunotoxin may also be administered via microspheres, liposomes or other microparticulate delivery systems placed in certain tissues including blood.

Dosages

In therapeutic applications, the dosages of compounds used in accordance with the invention vary depending on the class of compound and the condition being treated. The age, weight, and clinical condition of the recipient patient; and the experience and judgment of the clinician or practitioner administering the therapy are among the factors affecting the selected dosage. For example, the dosage of an immunoglobulin can range from about 0.1 milligram per kilogram of body weight per day to about 10 mg/kg per day for polyclonal antibodies and about 5% to about 20% of that amount for monoclonal antibodies. In such a case, the immunoglobulin can be administered once daily as an intravenous infusion.

Preferably, the dosage is repeated daily until either a therapeutic result is achieved or until side effects warrant discontinuation of therapy. Generally, the dose should be sufficient to treat or ameliorate symptoms or signs of MS without producing unacceptable toxicity to the patient.

An effective amount of the compound is that which provides either subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer. The dosing range varies with the compound used, the route of administration and the potency of the particular compound.

II. Obtention and isolation of MS inducing HHV-6 viral strains.

A. "Representational difference analysis."

This work is the result of recent developments in DNA amplification technology. These developments permit the identity of pathogens within a host without physical isolation of the intact pathogen. More specifically, these developments permit us to analyze the difference between two complex genomes to identify infectious agents by comparing non-infected tissue with infected tissue. Recently, a type of subtractive hybridization method which provides such ability to a certain extent was reported by Lisitsyn, Lisitsyn and Wigler in *Science* 259:946 (1993) and was termed "representational difference analysis" (RDA). Within certain limitations, RDA technology permits the selective amplification of DNA sequences that are present in one genome (tester) that are absent in another (driver). *Id.* It also makes use of the polymerase chain reaction (PCR) technology for amplifying nucleic acid.

In RDA, at the hybridization step, adaptors are present on the 5' ends of the nucleic acid fragments from the tester population and not present on fragments from the driver population. The driver (in excess) and tester nucleic acid fragments are combined under hybridization conditions. After hybridization, only tester molecules that self-anneal contain adaptors on both ends, so that on subsequent PCR, only these

molecules amplify from the complex reaction mix. Molecules in common between driver and tester form heteroduplexes lacking one priming site, so they are not exponentially amplified. The driver sequences contain no priming sites, so they are not amplified during PCR. In Lisitsyn *et al.*, *supra*, high molecular weight DNA of the same genotype in the tester and driver was used, with spiked model pathogens in the tester (adenovirus-2, bacteriophage lambda).

One of the limitations of RDA is that it is necessary to compare DNA from the same individual, or possibly from an identical twin or from both of the parents. See, Lisitsyn *et al.*, supra at page 949. The RDA proponents stated that, "[t]hese DNA's [tester and driver DNA] cannot derive from unrelated individuals, as the abundant polymorphic differences in their DNA's would obscure the detection of the pathogen." *Id.* Although RDA was developed using the same genotype as driver and tester, in many cases with human samples, constitutional non-diseased tissue cannot be obtained simultaneously with diseased tissue from the same patient. Even when constitutional DNA can be obtained from the same patient, there is always the possibility, particularly with viruses that can enter the systemic circulation, that the pathogen is present in many of the patient's non-diseased tissues. This creates a problem during the RDA procedure, because the driver would then contain the same sequence that one was attempting to isolate from the tester, and the sequence might be subtracted out. Thus, the RDA process is not well suited for identification of pathogens.

This invention is based upon an improvement of the above technology where a pooled nucleic acid driver is hybridized against one or more individual testers. Bands on a gel representing nucleic acid fragments which differ between the individuals are more likely to be polymorphic amplifiable restriction fragments (PARFs), and bands which are in common between infected individuals are much more likely to be markers or pathogens that are present in common between several tester samples. In previously described methods, the large number of PARFS which are produced that are specific for

each individual tester would make the identification of only products that are in common between multiple individuals extremely difficult.

"Healthy" driver and tester (with suspected pathogens) nucleic acid sources are each separately fragmented (e.g., endonucleases) and linked to adaptors which can be hybridized to PCR primers. The driver and tester are amplified to produce amplicons. The adaptors are removed from the amplicons of the both tester and driver fragments and new adaptors are ligated to the 5' ends of the testers amplicon fragments which are then combined with the driver fragments present in excess. The tester and driver amplicons are denatured and permitted to reanneal. The pathogenic DNA can only anneal to itself and is the only amplifiable material in the sample.

B. Isolation of the Herpesvirus associated with MS.

Using conventional methods, HHV-6 can be propagated *in vitro*. MSV-1206 and other like strains inducing MS can be propagated by the standard techniques for growing HHV-6 strains (Ablashi, D.V., 1991, *et al. Virology 184*:545-552). Briefly, PHA stimulated cord blood mononuclear cells, macrophage, neuronal, or glial cell lines are cocultivated with cerebrospinal fluid, plasma, peripheral blood leukocytes, or tissue extracts containings viral infected cells or purified virus. The recipient cells are treated with 5 μg/ml polybrene for 2 hours at 37° C prior to infection. Infected cells are observed by demonstrating morphological changes, as well as being positive for HHV-6 antigens using HHV-6 monoclonal antibodies in an immunofluorescence assay.

For virus isolation, the virus is either harvested directly from the culture fluid by direct centrifugation, or the infected cells are harvested, homogenized or lysed and the virus is separated from cellular debris and purified by standard methods of isopycnic sucrose density gradient centrifugation.

III. Detection and diagnosis of HHV-6 associated with MS.

The detection of the herpesvirus and the diagnosis of virus associated MS are essentially identical processes. The basic principle is to detect the virus using specific ligands that bind to the virus but not to other proteins or nucleic acids in a normal human cell or its environs. The ligands can either be nucleic acid or antibodies. The ligands can be naturally occurring or genetically or physically modified such as nucleic acids with non-natural or antibody derivatives, i.e., Fab or chimeric antibodies.

Samples from patients with MS can be taken wherein the virus or evidence of the virus is suspected (*e.g.*, antibodies to the virus) or where the nucleic acid to be detected is likely to be resident. Typically the samples are taken from blood (cells, serum and/or plasma), cerebrospinal fluid (CSF), or, most preferably, from tissue in the brain. An intrathecal catheter would be a convenient means to sample and monitor virus. The most accurate diagnosis for MS will occur if elevated titers of the virus are detected in the brain or CSF, which is the focal point of the disease. The detection of high titers of the virus in the brain is by itself indicative of the disease, though confirmation of other MS diagnostic factors are also desirable such as those already known. As mentioned, MS is also indicated if the indicative nucleic acid sequence (as opposed to antibodies) is detected in samples from other tissue locations. MS is further indicated if antibodies to the virus are detected and at least one of the other diagnostic factors for MS is present.

The invention can be used in conjunction with other tests to corroborate the diagnosis of MS. For example, application of the invention in addition to certain laboratory parameters which are generally associated with MS can substantiate the diagnosis. Specifically, the finding of elevated immunoglobulin levels in the CSF is diagnostically corroborative and can be used in conjunction with the invention to aid the clinician's assessment. Frequently, such immunoglobulins demonstrate an oligoclonal banding pattern.

Other diagnostic parameters include MRI of the central nervous system, which can reveal lesions presumably due to demyelination, and the visual evoked response, which may show a decreased initial amplitude and a prolonged latency of the major positive peak. Further examples of tests which can corroborate the diagnosis of MS include those that detect decreased suppressor or cytotoxic T cell activity and antibodies to myelin basic proteins.

The detection and assay of viral titer is also useful for measuring the progression of the disease or the effect of treatment. By measuring viral titers at different points, one can assess the progress or treatment of the disease.

MS is known to vary in frequency by geographic location. We can therefore expect that different substrains of HHV-6 that cause MS will differ in nucleotide sequences detectable as markers, as well as in pathogenicity. Included are markers that identify the substrains that cause MS in the Los Angeles, California population, which are typified by MSV-1206.

Because there are different substrains of HHV-6*n*, it is useful to identify, study and classify these substrains. Any strain of HHV-6, including variants described here and elsewhere, may be detected for in general assays, including those useful to discriminate between type A and type B, group 1 and group 2. Several HHV substrains have been described. See, for example, Aubin *et al.*, 1991, *J. Clin. Microbiol.* 29:367 and Luppi *et al.*, 1993, *JID* 168:1074.

Preferably and for the most accurate diagnosis, the assay is performed on samples taken during periods of active disease as opposed to periods of remission, including from such sources as CSF, brain biopsies or peripheral blood.

A. Nucleic Acid Assays.

Preferably, the diagnostic assays utilize nucleic acid assays such as nucleic acid hybridization assays and assays which detect amplification of specific nucleic acid to detect for a nucleic acid sequence of HHV-6^n, and particularly HHV-6B2 strains by detecting for one or more of the markers

described herein and/or sequences set out in Table 1. A preferred marker is named MDBP providing a HindIII marker in the DNA binding protein gene. Sequences homologous to those, as described below, will be understood to be effective as well. Marker MDPB was found in 10/27 MS samples and 2/26 control samples.

Table 1. Genetic Markers for HHV-6 associated MS

1. Name: RF1
   External Primers:
      33A1:5'-CGAAAAGACGTTGGACAATC-3' Seq. ID. No. 1
      33A2:5'-AAGAGACAGTCGATGTGATC-3' Seq. ID. No. 2
   Internal Primers:
      33A3:5'-ACGTTCACGAACCTCGATA-3'Seq. ID. No. 3
      33A4:5'-AGATGGCTCTCTGTTGTTC-3'Seq. ID. No. 4
   Polymorphism: TaqI site
      MS: actgtcatccagcaggtatAgaaacgagaagctcatgctt Seq. ID. No. 5
      Con:actgtcatccagcaggtatCgaaacgagaagctcatgctt Seq. ID. No. 6

2. Name: VEHL
   External Primers:
      33B4:5'-TGGAGATGTTTGGTAGTTC-3'Seq. ID. No. 7
      33B5:5'-GAAACACTTCCATGAATTCG-3'Seq. ID. No. 8
   Internal Primers:
      33B6:5'-CGGAACGATGCCTTC-3' Seq. ID. No. 9
      33B7:5'-GAAGATATTGAACGAGA-3' Seq. ID. No. 10
   Polymorphism:
      MS: ctaattttattgacaaactCggggaaactcccgcacttcc Seq. ID. No. 11
      Con:ctaattttattgacaaactTggggaaactcccgcacttcc Seq. ID. No. 12

2. Name: MDBP
   External Primers:
      HHV1:5'-CTATCCCTCATCACCTCAGC-3' Seq. ID. No. 13
      HHV6:5'-GTGGTGTTAGACAAGCAGCC-3' Seq. ID. No. 14
   Internal Primers:
      MSHind9:5'-TAATTGTCTCACGCCGAACC-3' Seq. ID. No. 15
      HV16:5'-CTGTGATTGCGTCACTGTCC-3' Seq. ID. No. 16
   Polymorphism: HindIII
      MS: tattccctattagccaagctTacaaatttctctaatgtcaa Seq. ID. No. 17
      Con:tattccctattagccaagctCacaaatttctctaatgtcaa Seq. ID. No. 18

3. Name: 38An
   External Primers:
      38A5:5'-GGGTACGACTTGAGACG-3' Seq. ID. No. 19

38A8:5'-CCATCGACTTCAAACACC-3' Seq. ID. No. 20
Internal Primers:
38A7:5'-CTGAAAATGATCCTTCGTG-3' Seq. ID. No. 21
38A6:5'-ATCTTTACCGAACCATGATC-3' Seq. ID. No. 22
Polymorphism:
MS: gctgttaaattcacgttaaCagagcaagttcttcgaggt Seq. ID. No. 23
Con: gctgttaaattcacgttaaAagagcaagttcttcgaggt Seq. ID. No. 24

4. Name: 38A
External and Internal Primers: Same as 38An
Polymorphism:
MS: tagaatttcaatatttaattTcacatgtaatttaagcattt Seq. ID. No. 25
Con: tagaatttcaatatttaattCcacatgtaatttaagcattt Seq. ID. No. 26

5. Name: 38E
External Primers:
38E1:5'-TCTATGCGTGATGGAACG-3' Seq. ID. No. 27
38E8:5'-TCTCATCTTGTGGAGCAC-3' Seq. ID. No. 28
Internal Primers:
38E3:5'-ACGATCATGTTGTGAGCG-3' Seq. ID. No. 29
38E6:5'-AAATGCCAGTCCAACAGC-3' Seq. ID. No. 30
Polymorphism:
MS: tgctttaatctccggaaatgTtgtgatatggcgcgatttag Seq. ID. No. 31
Con:tgctttaatctccggaaatgCtgtgatatggcgcgatttag Seq. ID. No. 32

6. 38EP
External Primers:
38E2:5'-ATGCAACATCAGCGGATG-3' Seq. ID. No. 52
38E7:5'-GTGAAAGAACGAAGGACG-3' Seq. ID. No. 53
Internal Primers:
38E4:5'-TACGAGACTAGGATAGCG-3' Seq. ID. No. 54
38E5:5'-TTTGCTGTTGGACTGGC-3' Seq. ID. No. 80
Polymorphism: PvuII
MS: tgttgtctgttcaggcagctGaaatccgaaactcttccaaa Seq. ID. No. 33
Con:tgttgtctgttcaggcagctAaaatccgaaactcttccaaa Seq. ID. No. 34

7. 38D
External Primers:
38D5:5'-TATGGCCTATTGCATGGC-3' Seq. ID. No. 35
38D2:5'-AAACACCTATGGTTCGCC-3' Seq. ID. No. 36
Internal Primers:
38D7:5'-CATGTGATGATCACACCG-3' Seq. ID. No. 37
38D4:5'-AATCAGCAAAGAGCCGAG-3' Seq. ID. No. 38
Polymorphism: Bst711
MS: gaaatgtttcncgtgtacgcGgcgcgaaaaggggtcggatt Seq. ID. No. 39
Con:gaaatgtttcncgtgtacgcAgcgcgaaaaggggtcggatt Seq. ID. No. 40

8. UGL

External Primers:

43A1:5'-CTGCAGGATAACCCAGAGC-3' Seq. ID. No. 41

43A2:5'-CTTCATCCTGAACGTGATCT-3' Seq. ID. No. 42

Internal Primers:

43A3:5'-TACGCACATTGTTTGCGC-3' Seq. ID. No. 43

43A4:5'-ATCCACTGTAGTAGGGC-3' Seq. ID. No. 44

Polymorphism: NlaIV

MS: aggcatcacaaaaattatcagAttccctttaactctttgactctc Seq. ID. No. 45

Con:aggcatcacaaaaattatcagGttccctttaactctttgactctc Seq. ID. No. 46

The prototype neurotropic HHV-6 is referred to herein as MSV-1206. MSV-1206 is a herpes-like agent identified herein which contains nucleic acid sequences representing Sequence ID Nos. 17 (MDPB), 25 (38A), 31 (38E), 33 (38EP), 39 (38D,BST711) and 45 (UGL, UrGly Nla1). It is a member of a subset of the HHV-6B group 2 subfamily of HHV-6 viruses. Those of skill in the art will appreciate that additional portions of the sequences for MSV-1206 can be readily obtained by designing a primer to the described sequence here and using it in combination with a random primer to walk out from the known sequence and obtain sequences which are heretofore undescribed. See, for example, Parker and Burmer, *Nuc. Acids. Res.* 19(11):3055 (1991), incorporated by reference herein. Alternatively, one could screen a genomic library from MS diseased tissue with a probe designed from the described sequences.

It will be readily understood by those of skill in the art and it is intended here, that when reference is made to particular sequence listings, such as Sequence ID No. 1, such reference includes sequences which substantially correspond to its complementary sequence and those described including allowances for minor sequencing errors, single base changes, deletions, substitutions and the like, such that any such sequence variation corresponds to the nucleic acid sequence of the pathogenic organism or disease marker to which the relevant sequence listing relates.

By a nucleic acid sequence "homologous to" or "complementary to", it is meant a nucleic acid that selectively hybridizes, duplexes or binds to viral DNA

sequences encoding proteins or portions thereof when the DNA sequences encoding the viral protein are present in a human genomic or cDNA library. A DNA sequence which is homologous to a target sequence can include sequences which are shorter or longer than the target sequence so long as they meet the functional test set forth. Hybridization conditions are specified along with the source of the cDNA library. Typically, the hybridization is done in a Southern blot protocol using a 0.2XSSC, 0.1% SDS, 65°C wash. The term "SSC" refers to a citrate-saline solution of 0.15 M sodium chloride and 20 mM sodium citrate. Solutions are often expressed as multiples or fractions of this concentration. For example, 6XSSC refers to a solution having a sodium chloride and sodium citrate concentration of 6 times this amount or 0.9 M sodium chloride and 120 mM sodium citrate. 0.2XSSC refers to a solution 0.2 times the SSC concentration or 0.03 M sodium chloride and 4 mM sodium citrate.

Accepted means for conducting hybridization assays are known and general overviews of the technology can be had from a review of: *Nucleic Acid Hybridization: A Practical Approach*, Ed. Hames, B.D. and Higgins, S.J., IRL Press, 1985; *Hybridization of Nucleic Acids Immobilized on Solid Supports*, Meinkoth, J. and Wahl, G.; *Analytical Biochemistry*, Vol 238, 267-284, 1984 and Innis *et al.*, *PCR Protocols*, *supra*, all of which are incorporated by reference herein.

If PCR is used, primers are designed to target a specific portion of the nucleic acid of the targeted agent. For example, primers for amplifying a subsequence for MSV-1206, including any of the subsequences having one of the genetic markers identified herein or as set out in Sequence ID Nos. 1, 14, 24 and 25, would be effective. Preferred primers are those set out in Table 1. Preferably the primers are about 14 to about 24 nucleotides in length. From the information provided herein, those of skill in the art will be able to select appropriate specific primers.

Target specific probes may be used in the nucleic acid hybridization diagnostic assays for MS. The probes are specific for or complementary to the target of interest. For

example, probes to one of the nucleic acid sequences for MSV-1206 would be effective. For precise allelic differentiations the probes should be about 14 nucleotides long and preferably about 20-30 nucleotides. For more general detection of HHV-6, nucleic acid probes are about 50 to about 1000 nucleotides, most preferably about 200 to about 400 nucleotides.

A sequence is "specific" for a target organism of interest if it includes a nucleic acid sequence which when detected is determinative of the presence of the organism in the presence of a heterogeneous population of proteins and other biologics. A specific nucleic acid probe is targeted to that portion of the sequence which is determinative of the organism and will not hybridize to other sequences especially those of the host where a pathogen is being detected.

The specific nucleic acid probe can be RNA or DNA polynucleotide or oligonucleotide, or their analogs. The probes may be single or double stranded nucleotides. The probes of the invention may be synthesized enzymatically, using methods well known in the art (*e.g.*, nick translation, primer extension, reverse transcription, the polymerase chain reaction, and others) or chemically (*e.g.*, by methods such as the phosphoramidite method described by Beaucage and Carruthers, *Tetrahedron Lett.* 22:1859-1862 (1981), or by the triester method according to Matteucci, *et al.*, *J. Am. Chem. Soc.*, 103:3185 (1981), both incorporated herein by reference).

The probe must be of sufficient length to be able to form a stable duplex with its target nucleic acid in the sample, *i.e.*, at least about 14 nucleotides, and may be longer (*e.g.*, at least about 50 or 100 bases in length). Often the probe will be more than about 100 bases in length. For example, when probe is prepared by nick-translation of DNA in the presence of labeled nucleotides the average probe length may be about 100-600 bases.

As noted above, the probe will be capable of specific hybridization to a specific MS viral nucleic acid. Such "specific hybridization" occurs when a probe hybridizes to a target nucleic acid, as evidenced by a detectable signal,

under conditions in which the probe does not hybridize to other nucleic acids (*e.g.*, animal cell or other bacterial nucleic acids) present in the sample. A variety of factors including the length and base composition of the probe, the extent of base mismatching between the probe and the target nucleic acid, the presence of salt and organic solvents, probe concentration, and the temperature affect hybridization, and optimal hybridization conditions must often be determined empirically. For discussions of nucleic acid probe design and annealing conditions, see, for example, Sambrook, *supra*, *Current Protocols in Molecular Biology*, F. Ausubel *et al.*, ed. Greene Publishing and Wiley-Interscience, New York (1987) [hereinafter referred to as Sambrook], Methods in Enzymology Vol. 152, Berger, S. and Kimmel, A. ed. Academic Press, New York (1987) or Tijssen, J. *Hybridization with Nucleic Acid Probes* pp. 495-524, Elsevier, Amsterdam (1993) all of which are incorporated herein by reference.

Usually, at least a part of the probe will have considerable sequence identity with the target nucleic acid. Although the extent of the sequence identity required for specific hybridization will depend on the length of the probe and the hybridization conditions, the probe will usually have at least 70% identity to the target nucleic acid, more usually at least 80% identity, still more usually at least 90% identity and most usually at least 95% or 100% identity.

A probe can be identified as capable of hybridizing specifically to its target nucleic acid by hybridizing the probe to a sample treated according the protocol of this invention where the sample contains both target virus and animal cells (*e.g.*, nerve cells). A probe is specific if the probe's characteristic signal is associated with HHV-6^{n} DNA in the sample and not generally with the DNA of the host cells and non-biological materials (*e.g.*, substrate) in a sample.

The following stringent hybridization and washing conditions will be adequate to distinguish a specific probe (*e.g.*, a fluorescently labeled DNA probe) from a probe that is not specific: incubation of the probe with the sample for 12 hours at 37°C in a solution containing denatured probe, 50%

formamide, 2X SSC, and 0.1% (w/v) dextran sulfate, followed by washing in 1X SSC at 70°C for 5 minutes; 2X SSC at 37°C for 5 minutes; 0.2X SSC at room temperature for 5 minutes, and H_2O at room temperature for 5 minutes. Those of skill will be aware that it will often be advantageous in nucleic acid hybridizations (*i.e., in situ*, Southern, or other) to include detergents (*e.g.*, sodium dodecyl sulfate), chelating agents (*e.g.*, EDTA) or other reagents (*e.g.*, buffers, Denhardt's solution, dextran sulfate) in the hybridization or wash solutions.

To test the specificity of the virus specific probes, the probes can be tested on host cells containing the MS-associated virus and compared with the results from cells containing non MS-assocated virus.

It will be apparent to those of ordinary skill in the art that a convenient method for determining whether a probe is specific for a MS-associated viral nucleic acid utilizes a Southern blot (or Dot blot) using DNA prepared from one or more HHV-6 viruses. Briefly, to identify a target specific probe DNA is isolated from the virus such as described in Table 1 and tested against the host cells. Test DNA either viral or cellular is transferred to a solid (*e.g.*, charged nylon) matrix. The probes are labelled following conventional methods. Following denaturation and/or prehybridization steps known in the art, the probe is hybridized to the immobilized DNAs under stringent conditions. Stringent hybridization conditions will depend on the probe used and can be estimated from the calculated T_m (melting temperature) of the hybridized probe (see, *e.g.*, Sambrook for a description of calculation of the T_m). For radioactively-labeled DNA or RNA probes an example of stringent hybridization conditions is hybridization in a solution containing denatured probe and 5x SSC at 65°C for 8-24 hours followed by washes in 0.1x SSC, 0.1% SDS (sodium dodecyl sulfate) at 50-65°C. In general, the temperature and salt concentration are chosen so that the post hybridization wash occurs at a temperature that is about 5°C below the T_M of the hybrid. Thus for a particular salt concentration the

temperature may be selected that is 5°C below the T_M or conversely, for a particular temperature, the salt concentration is chosen to provide a T_M for the hybrid that is 5°C warmer than the wash temperature. Following stringent hybridization and washing, a probe that hybridizes to the MS-associated viral DNA but not to the non-MS associated viral DNA, as evidenced by the presence of a signal associated with the appropriate target and the absence of a signal from the non-target nucleic acids, is identified as specific for the MS associated virus.

It will be appreciated that in determining probe specificity and in utilizing the method of this invention to detect MS-associated HHV-6, a certain amount of background signal is typical and can easily be distinguished by one of skill from a specific signal. Two fold signal over background is acceptable.

A preferred method for detecting MS associated HHV-6 is the use of PCR and/or dot blot hybridization. The presence or absence of an MS causal agent as a diagnosis for MS includes Southern transfers, solution hybridization or non-radioactive detection systems, all of which are well known to those of skill in the art. Hybridization is carried out using probes. Visualization of the hybridized portions allows the qualitative determination of the presence or absence of the causal agent.

Similarly, a Northern transfer may be used for the detection of message in samples of RNA or reverse transcriptase PCR and cDNA can be detected by methods described above. This procedure is also well known in the art. See, Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. incorporated by reference herein.

A preferred method for diagnosing MS is to first determine if the person suspected of having MS is hosting a herpesvirus and then determining if the virus has any of the above described genetic markers associated with previous MS patients.

A preferred method of detecting for the presence or absence of the *Hind*III site found within the major DNA binding protein of MSV-1206 (See Figure 2 and the Example section below). This detection can be readily accomplished, for example, by using PCR primers that flank the MSV-1206 *Hind*III site, such as two of the primers provided in Sequence ID Nos. 13-16 to amplify a portion of the MSV-1206 sequence bearing that site in a patient sample. The amplified product can then be subjected to *Hind*III digestion. The products of the digestion can then be separated by, for example, gel electrophoresis to determine if fragments are obtained that correspond with those predicted if the *Hind*III site is present within an amplified product. The detection of the *Hind*III site within an amplified product then confirms or is indicative of MSV-1206 and MS.

An alternative means for determining the presence of MSV-1206 is *in situ* hybridization, or more recently, in situ polymerase chain reaction. In situ PCR is described in Neuvo *et al.*, 1993, Intracellular localization of polymerase chain reaction (PCR)-amplified Hepatitis C cDNA, *American Journal of Surgical Pathology, 17(7)*, 683-690; Bagasra *et al.*, 1992, Detection of Human Immunodeficiency virus type 1 provirus in mononuclear cells by in situ polymerase chain reaction *J. New England Journal of Medicine, 326(21)*, 1385-1391; and Heniford *et al.*, 1993, Variation in cellular EGF receptor mRNA expression demonstrated by in situ reverse transcriptase polymerase chain reaction, *Nucleic Acids Research 21(14)*, 3159-3166.. *In situ* hybridization assays are well known and are generally described in Angerer, *et al.*, 1987, *Methods Enzymol.* 152:649-660 incorporated by reference herein. In an *in situ* hybridization, cells are fixed to a solid support, typically a glass slide. The cells are then contacted with a hybridization solution at a moderate temperature to permit annealing of target-specific probes that are labelled. The probes are preferably labelled with radioisotopes or fluorescent reporters.

For diagnosis in blood samples, it is desirable to roughly quantify the viral copy number since sporadic reports state that HHV-6 may be present in the blood of normal patients at a very low level (as low as one infected lymphocyte/100,000). Cone *et al.*, 1993, *J. Clin. Micro.* 31:(5) 1262.

B. Immunoassays.

In addition to the detection of the causal agent using nucleic acid hybridization technology, one can use immunoassays to detect for the virus, specific peptides, or for antibodies to the virus or peptides. A general overview of the applicable technology is in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Pubs., Cold Spring Harbor, N.Y. (1988), incorporated by reference herein.

In one embodiment, antibodies to HHV-6 can be used to detect for the agent in the sample. In brief, to produce antibodies to the agent or peptides, the sequence being targeted is expressed in transfected cells, preferably bacterial cells, and purified. The product is injected into a mammal capable of producing antibodies. Either monoclonal or polyclonal antibodies (as well as any recombinant antibodies) specific for the gene product can be used in various immunoassays. Such assays include competitive immunoassays, radioimmunoassays, Western blots, ELISA, indirect immunofluorescent assays and the like. For competitive immunoassays, see Harlow and Lane (1988) at pages 567-573 and 584-589.

Monoclonal antibodies or recombinant antibodies may be obtained by various techniques familiar to those skilled in the art. Briefly, spleen cells or other lymphocytes from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (see, Kohler and Milstein, 1976, *Eur. J. Immunol.* 6:511-519, incorporated herein by reference). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened

for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. New techniques using recombinant phage antibody expression systems can also be used to generate monoclonal antibodies. See for example: McCafferty, J *et al.* 1990, *Nature, 348*:552; Hoogenboom, H.R. *et al.*, 1991, *Nuc. Acids Res. 19*:4133, 1991; and Marks, J.D. *et al.* 1991. *J. Mol Biol. 222*:581-597.

Monoclonal antibodies prepared against HHV-6 have been described, see for example, Balachandran *et al.*, 1989, *J. Virol.* 63:2835-2840. It is preferred, however, that monoclonal antibodies be raised to peptides derived from MSV-1206.

Such peptides may be produced by expressing the specific sequence in a recombinantly engineered cell such as bacteria, yeast, filamentous fungal, insect (especially employing baculoviral vectors), and mammalian cells. Those of skill in the art are knowledgeable in the numerous expression systems available for expression of herpes virus protein.

Briefly, the expression of natural or synthetic nucleic acids encoding viral protein will typically be achieved by operably linking the desired sequence or portion thereof to a promoter (which is either constitutive or inducible), and incorporated into an expression vector. The vectors are suitable for replication or integration in either prokaryotes or eukaryotes. Typical cloning vectors contain antibiotic resistance markers, genes for selction of transformants, inducible or regulatable promoter regions, and translation terminators that are useful for the expression of viral genes.

Methods for the expression of cloned genes in bacteria are also well known. In general, to obtain high level expression of a cloned gene in a prokaryotic system, it is advisable to construct expression vectors containing a strong promoter to direct mRNA transcription. The inclusion of selection markers in DNA vectors transformed in *E. coli* is

also useful. Examples of such markers include genes specifying resistance to antibiotics. See Sambrook, *et al.*, *supra*, for details concerning selection markers and promoters for use in *E. coli*. Suitable eukaryote hosts may include plant cells, insect cells, mammalian cells, yeast, and filamentous fungi.

Methods for characterizing naturally processed peptides bound to MHC (major histocompatibility complex) I molecules have been developed. See, Falk *et al.*, 1991, *Nature* 351:290 (1991), and PCT publication No. WO 92/21033 published November 26, 1992, both of which are incorporated by reference herein. Typically, these methods involve isolation of MHC class I molecules by immunoprecipitation or affinity chromatography from an appropriate cell or cell line. Other methods involve direct amino acid sequencing of the more abundant peptides in various HPLC fractions by known automatic sequencing of peptides eluted from Class I molecules of the B cell type (Jardetzkey, *et al.*, 1991, *Nature* 353:326 (1991), incorporated by reference herein, and of the human MHC class I molecule, HLA-A2.1 type by mass spectrometry (Hunt, 1992, *et al.*, *Science* 225:1261, incorporated by reference herein). See also, Rötzschke and Falk, 1991, *Immunol. Today* 12:447, incorporated by reference herein for a general review of the characterization of naturally processed peptides in MHC class I. Further, Marloes, *et al.*, 1991, *Eur. J. Immunol.* 21:2963-2970 (1991), incorporated by reference herein, describe how class I binding motifs can be applied to the identification of potential viral immunogenic peptides *in vitro*.

The peptides described herein produced by recombinant technology may be purified by standard techniques well known to those of skill in the art. Recombinantly produced viral sequences can be directly expressed or expressed as a fusion protein. The protein is then purified by a combination of cell lysis (*e.g.*, sonication) and affinity chromatography. For fusion products, subsequent digestion of the fusion protein with an appropriate proteolytic enzyme releases the desired peptide.

The purified viral protein or other peptides when described as "isolated" or "substantially pure" describes a protein that has been separated from components which naturally accompany it. Typically, a monomeric protein is substantially pure when at least about 85% or more of a sample exhibits a single polypeptide backbone. Minor variants or chemical modifications may typically share the same polypeptide sequence. Depending on the purification procedure, purities of 85%, and preferably over 95% pure are possible. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band on a polyacrylamide gel upon staining. For certain purposes high resolution will be needed and HPLC or a similar means for purification utilized.

The proteins may be purified to substantial purity by standard techniques well known in the art, including selective precipitation with such substances as ammonium sulfate, column chromatography, immunopurification methods, and others. See, for instance, R. Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag, New York (1982), incorporated herein by reference.

The antibodies raised against the viral strain or peptides may be detectably labelled, utilizing conventional labelling techniques well-known to the art. Thus, the antibodies may be radiolabelled using, for example, radioactive isotopes such as ^{3}H, ^{125}I, ^{131}I, and ^{35}S.

The antibodies may also be labelled using fluorescent labels, enzyme labels, free radical labels, or bacteriophage labels, using techniques known in the art. Typical fluorescent labels include fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, alophycocyanin, and Texas Red.

Since specific enzymes may be coupled to other molecules by covalent links, the possibility also exists that they might be used as labels for the production of tracer materials. Suitable enzymes include alkaline phosphatase, beta-galactosidase, glucose-6-phosphate dehydrogenase, maleate

dehydrogenase, and peroxidase. Two principal types of enzyme immunoassay are the enzyme-linked immunosorbent assay (ELISA), and the homogeneous enzyme immunoassay, also known as enzyme-multiplied immunoassay (EMIT, Syva Corporation, Palo Alto, CA). In the ELISA system, separation may be achieved, for example, by the use of antibodies coupled to a solid phase. The EMIT system depends on deactivation of the enzyme in the tracer-antibody complex; the activity can thus be measured without the need for a separation step.

Additionally, chemiluminescent compounds may be used as labels. Typical chemiluminescent compounds include luminol, isoluminol, aromatic acridinium esters, imidazoles, acridinium salts, and oxalate esters. Similarly, bioluminescent compounds may be utilized for labelling, the bioluminescent compounds including luciferin, luciferase, and aequorin.

Once labeled, the antibody may be employed to identify and quantify immunologic counterparts (antibody or antigenic polypeptide) utilizing techniques well-known to the art.

A description of a radioimmunoassay (RIA) may be found in *Laboratory Techniques in Biochemistry and Molecular Biology* by Work, T.S. *et al.*, with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, T., North Holland Publishing Company, New York (1978), incorporated by reference herein.

A description of general immunometric assays of various types can be found in the following U.S. Pat. Nos. 4,376,110 (David *et al.*) or 4,098,876 (Piasio).

Alternatively, hemagglutination Inhibition (HI) and Complement Fixation (CF) which are two laboratory tests that can be used to detect infection with HHV by testing for the presence of antibodies against the virus or antigens of the virus.

The serological methods are useful when one wishes to detect antibody to a specific variant. For example, one may wish to see how well a vaccine recipient has responded to the new variant. Alternatively, one may take serum from a

patient to see which variant the patient responds to the best. The CF and ELISA tests, in the present standard configurations, are designed to detect conserved antigens and cannot differentiate between variants. The serological tests most applicable here are HI and microneutralization.

IV. Vaccines and Prophylaxis for MS.

A. Vaccines.

The invention also provides substances suitable for use as vaccines for the prevention of MS and methods for administering them. The vaccines are directed against HHV-6, and most preferably, neurotropic HHV-6 such as MSV-1206. Preferably, the vaccines comprise HHV-6 antigen, most preferably peptides encoded by a sequence of a neurotropic HHV-6.

Vaccines can be made recombinantly. Typically, a vaccine will include from about 1 to about 50 micrograms of antigen or antigenic protein or peptide. More preferably, the amount of protein is from about 15 to about 45 micrograms. Typically, the vaccine is formulated so that a dose includes about 0.5 milliliters. The vaccine may be administered by any route known in the art. Preferably, the route is parenteral. More preferably, it is subcutaneous or intramuscular.

There are a number of strategies for amplifying an antigen's effectiveness, particularly as related to the art of vaccines. For example, cyclization or circularization of a peptide can increase the peptide's antigenic and immunogenic potency. See U.S. Pat. No. 5,001,049 which is incorporated by reference herein. More conventionally, an antigen can be conjugated to a suitable carrier, usually a protein molecule. This procedure has several facets. It can allow multiple copies of an antigen, such as a peptide, to be conjugated to a single larger carrier molecule. Additionally, the carrier may possess properties which facilitate transport, binding, absorption or transfer of the antigen.

For parenteral administration, such as subcutaneous injection, examples of suitable carriers are the tetanus toxoid, the diphtheria toxoid, serum albumin and lamprey, or

keyhole limpet, hemocyanin because they provide the resultant conjugate with minimum genetic restriction. Conjugates including these universal carriers can function as T cell clone activators in individuals having very different gene sets.

The conjugation between a peptide and a carrier can be accomplished using one of the methods known in the art. Specifically, the conjugation can use bifunctional cross-linkers as binding agents as detailed, for example, by Means and Feeney, "A recent review of protein modification techniques," *Bioconjugate Chem.* 1:2-12 (1990).

Vaccines against a number of the Herpesviruses have been successfully developed. Vaccines against Varicella-Zoster Virus using a live attenuated Oka strain is effective in preventing herpes zoster in the elderly, and in preventing chickenpox in both immunocomprised and normal children (Hardy, I., *et al.*, *Inf. Dis. Clin. N. Amer.* 4(1):159 (1990); Hardy, I. *et al.*, *New Engl. J. Med.* 325 (22):1545 (1991); Levin, M.J. *et al.*, *J. Inf. Dis.* 166(2):253 (1992); Gershon, A.A., *J. Inf. Dis.* 166(Suppl):563 (1992). Vaccines against Herpes simplex Types 1 and 2 are also commercially available with some success in protection against primary disease, but have been less successful in preventing the establishment of latent infection in sensory ganglia (Roizman, B. *Rev. Inf. Disease*, 13 Suppl. 11:S892 (1991); Skinner, G.R. *et al.*, *Med. Microbiol. Immunol.* 180(6):305 (1992)).

Vaccines against HHV-6 can be made by isolating extracellular viral particles from infected cell cultures, inactivating the virus with formaldehyde followed by ultracentrifugation to concentrate the viral particles and remove the formaldehyde, and immunizing individuals with 2 or 3 doses containing 1 x 10^9 virus particles (Skinner, G.R. *et al.*, *Comp. Immuno. Microbiol. Inf. Dis.* 14(2):13 (1991)). Alternatively, envelope glycoproteins can be expressed in E. coli or transfected into stable mammalian cell lines, the proteins can be purified and used for vaccination (Lasky, L.A., *J. Med. Virol.* 31(1):59 (1990)). MHC - binding peptides

from HHV-6 infected cells can be identified for vaccine candidates per the methodology of Marloes *et al., supra*.

The antigen may be combined or mixed with various solutions and other compounds as is known in the art. For example, it may be administered in water, saline or buffered vehicles with or without various adjuvants or immunodiluting agents. Examples of such adjuvants or agents include aluminum hydroxide, aluminum phosphate, aluminum potassium sulfate (alum), beryllium sulfate, silica, kaolin, carbon, water-in-oil emulsions, oil-in-water emulsions, muramyl dipeptide, bacterial endotoxin, lipid X, Corynebacterium parvum (Propionibacterium acnes), Bordetella pertussis, polyribonucleotides, sodium alginate, lanolin, lysolecithin, vitamin A, saponin, liposomes, levamisole, DEAE-dextran, blocked copolymers or other synthetic adjuvants. Such adjuvants are available commercially from various sources, for example, Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.) or Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Michigan). Other suitable adjuvants are Amphigen (oil-in-water), Alhydrogel (aluminum hydroxide), or a mixture of Amphigen and Alhydrogel. Only aluminum is approved for human use.

The proportion of antigen and adjuvant can be varied over a broad range so long as both are present in effective amounts. For example, aluminum hydroxide can be present in an amount of about 0.5% of the vaccine mixture (Al_2O_3 basis). On a per-dose basis, the amount of the antigen can range from about 0.1 μg to about 100 μg protein per patient. A preferable range is from about 1 μg to about 50 μg per dose. A more preferred range is about 15 μg to about 45 μg. A suitable dose size is about 0.5 ml. Accordingly, a dose for intramuscular injection, for example, would comprise 0.5 ml containing 45 μg of antigen in admixture with 0.5% aluminum hydroxide. After formulation, the vaccine may be incorporated into a sterile container which is then sealed and stored at a low temperature, for example 4°C, or it may be freeze-dried. Lyophilization permits long-term storage in a stabilized form.

The vaccines may be administered by any conventional method for the administration of vaccines including oral and parenteral (*e.g.*, subcutaneous or intramuscular) injection. Intramuscular administration is preferred. The treatment may consist of a single dose of vaccine or a plurality of doses over a period of time. It is preferred that the dose be given to a human patient within the first 8 months of life. The antigen of the invention can be combined with appropriate doses of compounds including influenza antigens, such as influenza type A antigens. Also, the antigen could be a component of a recombinant vaccine which could be adaptable for oral administration.

Vaccines of the invention may be combined with other vaccines for other diseases to produce multivalent vaccines. A pharmaceutically effective amount of the antigen can be employed with a pharmaceutically acceptable carrier such as a protein or diluent useful for the vaccination of mammals, particularly humans. Other vaccines may be prepared according to methods well-known to those skilled in the art.

Those of skill will readily recognize that it is only necessary to expose a mammal to appropriate epitopes in order to elicit effective immunoprotection. The epitopes are typically segments of amino acids which are a small portion of the whole protein. Using recombinant genetics, it is routine to alter a natural protein's primary structure to create derivatives embracing epitopes that are identical to or substantially the same as (immunologically equivalent to) the naturally occurring epitopes. Such derivatives may include peptide fragments, amino acid substitutions, amino acid deletions and amino acid additions within the amino acid sequence for a virus from HHV-6. For example, it is known in the protein art that certain amino acid residues can be substituted with amino acids of similar size and polarity without an undue effect upon the biological activity of the protein. HHV-6 proteins have significant tertiary structure and the epitopes are usually conformational. Thus, modifications should generally preserve conformation to produce a protective immune response.

B. Antibody Prophylaxis.

Therapeutic, intravenous, polyclonal or monoclonal antibodies can been used as a mode of passive immunotherapy of herpesviral diseases including perinatal varicella and CMV. Immune globulin from persons previously infected with HHV-6 and bearing a suitably high titer of antibodies against HHV-6 can be given in combination with antiviral agents (*e.g.* ganciclovir), or in combination with other modes of immunotherapy that are currently being evaluated for the treatment of MS, which are targeted to modulating the immune response (i.e. treatment with copolymer-1, antiidiotypic monoclonal antibodies, T cell "vaccination"). Antibodies to HHV-6, can be administered. Antibodies specific for an epitope expressed on HHV-6 infected cells are preferred and can be obtained as described above. See also I.B above.

V. Screening Assays For Pharmaceutical Agents of Interest in Alleviating the Symptoms of MS.

Since the causal agent of MS has been identified and described here, assays directed to identifying potential pharmaceutical agents that inhibit the biological activity of the agents are possible. MS drug screening assays which determine whether or not a drug has activity against HHV-6, and more particularly MSV-1206 are contemplated in this invention. Such assays comprise incubating a compound to be evaluated for use in MS treatment with cells which express MS associated HHV-6 viral proteins or peptides and determining therefrom the effect of the compound on the activity of such agent. *In vitro* assays in which the virus is maintained in suitable cell culture are preferred, though *in vivo* animal models would also be effective. Example 4 is typical of these *in vitro* assays.

Compounds with activity against the agent of interest or peptides from such agent can be screened in *in vitro* as well as *in vivo* assay systems. *In vitro* assays include infecting peripheral blood leukocytes or susceptible T cell lines such as MT-4 with the agent of interest in the presence of varying concentrations of compounds targeted

against viral replication, including nucleoside analogs, chain terminators, antisense oligonucleotides and random polypeptides (Asada, H. *et al.*, 1989, *J. Clin. Microbiol.* 27(10):2204; Kikuta *et al.*, 1989, *Lancet* Oct. 7:861 both incorporated by reference herein). Infected cultures and their supernatants can be assayed for the total amount of virus including the presence of the viral genome by quantitative PCR, by dot blot assays, or by using immunologic methods. For example, a culture of susceptible cells could be infected with HHV-6 in the presence of various concentrations of drug, fixed on slides after a period of days, and examined for viral antigen by indirect immunofluorescence with monoclonal antibodies to viral peptides (Kikuta *et al. supra.* Alternatively, chemically adhered MT-4 cell monolayers can be used for an infectious agent assay using indirect immunofluorescent antibody staining to search for focus reduction (Higashi, K. *et al.*, 1989, *J. Clin. Micro.* 27(10):2204, incorporated by reference herein).

As an alternative to whole cell *in vitro* assays, purified enzymes isolated from HHV-6 can be used as targets for rational drug design to determine the effect of the potential drug on enzyme activity, such as thymidine phosphotransferase or DNA polymerase. The genes for these two enzymes are provided herein. A measure of enzyme activity indicates effect on the agent itself.

Drug screens using herpes viral products are known and have been previously described in EP 0514830 (herpes proteases) and WO 94/04920 (U_L13 gene product).

VI. Other Embodiments.

HHV-6 isolates, previously reported, can be assigned to one of two groups on the basis of the following criteria (*Arch. Virol* 129:363, 1993): 1) all HHV-6 isolates are tropic for CD4+ T lymphocytes, but the group characterized by strains GS and U1102 (variant A) or by strain Z29 (variant B) may preferentially infect different cell lines; 2) each group has a distinct pattern of reactivity to monoclonal antibodies; 3) the two groups have characteristic restriction endonuclease sites due to restriction site polymorphisms that extend across

the entire genome; and 4) genomic sequence analysis of eight different regions of the genome revealed sequence identity ranging from 97-100% within groups and from 94-96% between groups, and predicted amino acid sequences from 98-100% within groups and 92-96% between groups. In addition, all HHV-6 sequences isolated from infants with exanthema subitem and related febrile illnesses have been Z29-like, with the exception of two patients from whom both types were isolated, and HHV-6 isolated from immunocompromised adults may belong to either or both groups. We have found that the vast majority of individuals are in fact infected with HHV-6 Type B2 strains, and that these viruses can be further subtyped by molecular markers into strains that are associated with MS. The MSV-1206 virus is a prototype for the MS associated virus that is found in the Los Angeles population.

For the reasons described herein, the partial nucleic acid sequences for MSV-1206 and other MS associated HHV-6 as set out in Table 1 are unique and are particularly useful for, *inter alia*, diagnosis of MS. Thus, the MSV-1206 sequences and the unique peptides they encode in substantially pure form are embodiments of this invention.

It will be understood by those of skill in the art that portions of each of these sequences greater than 14 nucleotides in length which are complementary to sequences (and corresponding peptides) found in MS patients and not complementary to sequences found in healthy individuals are included in the invention.

This invention further embraces diagnostic kits for detecting the presence of an MS agent in tissue samples, such as CSF or serum, comprising a container containing anti-HHV-6 antibodies, and instructional material for performing the test. Alternatively, inactivated viral particles or peptides derived from MSV-1206 may be used in a diagnostic kit to detect for antibodies specific to MS associated virus.

Diagnostic kits for detecting the presence of an MS agent in tissue samples, such as brain, CSF or other affected tissue, comprising a container containing a nucleic acid sequence specific for neurotropic HHV-6 and instructional

material for detecting for MS are also included. A container containing nucleic acid primers to any one of such sequences is optionally included as is HHV specific antibodies described herein.

Definitions

The following definitions are further provided to explain their use in the specification:

"Antigen" refers to an entity or fragment thereof which can induce an immune response in a mammal. The term includes immunogens and regions responsible for antigenicity or antigenic determinants.

"Antigenic determinant" refers to a region of immunodominance.

"Binding agent" refers to either DNA, antibody or antibody-like agents that can specifically attach to HHV products in the presence of biological material from normal, healthy human cells and fluids. Such agents are "*Herpesviridae* specific".

"Complementary" means that one nucleic acid hybridizes selectively to, or specifically to another nucleic acid. Selectivity of hybridization exists when hybridization occurs that is more selective than total lack of specificity. Typically, selective hybridization will occur when there is at least about 55% identity over a stretch of at least 14-25 nucleotides, preferably at least about 65%, more preferably at least about 75%, and most preferably at least about 90%. See, M. Kanehisa, *Nucleic Acids Res.* 12:203 (1984), incorporated herein by reference.

"Effective" refers to agents that ameliorate the symptoms of the disease by impacting the virus directly (titer) or by preventing the viral products from begining a cascade of metabolic events that lead to neurological degeneration.

"Isolated" or "substantially pure", when referring to nucleic acids, refers to those that have been purified away from other chromosomal or extrachromosomal DNA or RNA by standard techniques, including alkaline/SDS treatment, CsCl

banding, column chromatography, and other techniques well known in the art or as otherwise described herein. See, F. Ausubel, *et al.*, ed., *Current Protocols in Molecular Biology*, Greene Publishing and Wiley-Interscience, New York (1987), incorporated herein by reference.

"Nucleic acids", as used herein, may be DNA or RNA and may be single- or double-stranded. Additionally, substantial nucleic acid sequence identity exists when a nucleic acid segment will hybridize, under stringent hybridization conditions, to a complement of another nucleic acid strand.

"Nucleic acid probes" may be RNA or DNA fragments prepared, for example, by digesting plasmid nucleic acid, or by PCR, or synthesized by methods such as the phosphoramidite method described by Beaucage and Carruthers, *Tetrahedron Lett.* 22:1859-1862 (1981), or by the triester method according to Matteucci, *et al.*, *J. Am. Chem. Soc.* 103:3185 (1981), both incorporated herein by reference. Where a specific nucleic acid sequence is given, it is understood that the complementary strand is also identified and included, for the complementary strand will work equally well in situations where the target is a double-stranded nucleic acid.

A nucleic acid probe is complementary to a target nucleic acid if it will anneal only to a single desired position on that target nucleic acid under stringent hybridization conditions, *e.g.* 1 x SSC at 65°. Proper annealing conditions depend, for example, upon a probe's length, base composition, and the number of mismatches and their position on the probe, and must often be determined empirically.

For discussions of nucleic acid probe design and annealing conditions, see, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd ed.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989) or *Current Protocols in Molecular Biology*, F. Ausubel *et al.*, ed., Greene Publishing and Wiley-Interscience, New York (1987), both of which are incorporated herein by reference.

"Nucleic acid sequence" refers to a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. It includes both self-replicating plasmids, infectious polymers of DNA or RNA and nonfunctional DNA or RNA. Unless otherwise stated, where a single nucleotide sequence is described, its complement is implicitly described.

"Specifically immunoreactive" refers to a binding reaction between an antibody and antigen which is selectively determinative of the presence of the antigen in the presence of a heterogeneous population of proteins and other biologics.

Examples

Example 1: Identification of HHV-6 Sequences in Multiple Sclerosis (MS).

Genomic analysis was performed using a DNA pool of 24 PBLs from normal blood donors as driver against brain tissue samples obtained from five patients with MS. The samples were obtained from patients who had died with a clinical diagnosis of MS, underwent autopsy and brain dissection. Sections of frozen white matter with macroscopically identifiable plaques were used for tester DNA extraction, and the plaques were dissected from these tissues with a sterile scalpel.

DNA was extracted from these tissues as described above, digested with the restriction endonuclease *Hind*III, ligated onto adaptors and amplified by PCR. After 20 cycles, aliquots of the amplicons from each patient were stored at -70°C and the remainder was used as tester in an RDA experiment. After four rounds of RDA, several bands were identified that were in common between several individuals. One of these products was a 347 base band that was cloned into Bluescript IIKS+ (Stratagene, La Jolla, CA) at the *Hind*III cloning site and multiple clones were sequenced to provide the consensus sequence shown in Table 2 and Sequence ID Nos. 47 and 48. These sequences are from the human Herpesvirus 6B major DNA binding protein region (6B Ka2L gene).

Table 2.

Sequence 47 Human Herpesvirus 6B Ka2L gene, DNA binding protein
Sequence 48 Clone MS-1

HindIII

Sequence 47 CAGTTTAAGCATTATGGATGCCGCTAAAGCTTCCCTACCATCCACAAAAAAAAGCATATC
Sequence 48 ************************************

120

Sequence 47 ATCCATAGTCGGCTTGTCCTCCCTATCCCTCATCACCTCAGCTATTATTAATAATTCTGG
Sequence 48 **

180

Sequence 47 ATCAATATCGTTAGTTAAATTCTCAACAATGCTGAGAACCTTGCCCTTGACCACTTCCGT
Sequence 48 **********************T***

240

Sequence 47 ATCAAACATAATTGTCTCACGCCGAACCTTTTTCACTATAACTTCTGAGAACTTTGTAGC
Sequence 48 **

300

Sequence 47 AATAATCGTTTTCTGCCTCATAAATCTAAAATCTTGCAATGCAGAAGAGGTAGGGTTTAA
Sequence 48 **

360

Sequence 47 GTTCCTATCCACGCCACTTCCACCTATCCAACCTAACTGGCCAAACTGAAAATATTCCCT
Sequence 48 **

HindIII

420

Sequence 47 ATTAGCCAAGCTCACAAATTTCTCTAATGTCAAACCAAATGTAACCAAAGATCGACTTGT
Sequence 48 ************T

Comparative searches against the GenBank/EMBL library of known sequences demonstrated that these DNA fragments were 98% homologous to the major DNA binding protein (KA2L) gene of Herpesvirus-6B. The virus bearing sequence ID. No. 48 were as previously undescribed and has been named MSV-1206 by us.

The oligonucleotides of Table 3 were designed from the KA2L gene sequence of HHV-6B in GeneBank/EMBL and used to screen for the presence of HHV-6B related sequences in the RDA derived amplicons from samples from five MS patients. One microliter of each amplicon (50 ng) was placed into a 50 microliter PCR reaction with 5 pg each of primer 1 and 4, 1 and 2, or 2 and 3, 50 mMol/L KCl, 2 mMol/L magnesium chloride, 2.5 U of Taq polymerase, 100 mMol/L deoxynucleoside triphosphates, and underwent 35 cycles of PCR at 1 min at 95°C (denaturation), 1 min at 54°C (annealing) and 1 min at 72°C (extension). Ten microliters of each product underwent electrophoresis in 2% (w/v) agarose in the presence of ethidium bromide and visualized by UV transillumination.

Three of the five samples showed visible bands of the expected size bands from each of the three primer pairs. To further identify these bands as related to HHV-6B, the products of PCR with primers HHV-6-1 and HHV-6-2 were probed with an internal primer (HHV-6-4) which was kinased with ^{32}P-ATP and polynucleotide kinase and added to one cycle of the PCR reaction in an oligomer extension "hot blot" assay (Parker and Burmer, 1991, *Biotechniques* 10(1):94, incorporated by reference herein). The bands were positive by the hot blot assay, further verifying their relation to HHV-6B.

Table 3. Sequences of Primers Used to Amplify and Detect HHV-6B Related Sequences

Primer	Sequence	
HHV-1	5'-CTATCCCTCATCACCTCAGC-3'	(Sequence ID No. 13)
HHV-2	5'-GGCCAGTTAGGTTGGATAGG-3'	(Sequence ID No. 49)
HHV-3	5'-TGAGAACCTTGCCCTTGACC-3'	(Sequence ID No. 50)
HHV-4	5'-TGGTCAAGGGCAAGGTTCTC-3'	(Sequence ID No. 51)

Example 2. Using PCR to diagnose HHV-6b related Multiple Sclerosis.

A brain tissue sample from a MS patient is isolated and the DNA isolated using the alkaline SDS method described

above. The MSV-1206 type virus is detected by amplification of the markers with the primers listed, followed by detection of the MSV-1206 dot blot format, in which the PCR product is transferred to a Nylon membrane and hybridized with a labeled internal oligonucleotide probe that is complementary to the sequence containing the nucleotides specific for the virus under high stringency hybridization and wash conditions (Dyson, N.J. Immobilization of nucleic aids and hybridization analysis. In *Essential Molecular Biology: A Practical Approach*, Vol. 2, Brown, T.A., ed. IRL Press at Oxford University press, Oxford, pp. 111-156, 1991 and Sambrook, J., Fritsch, E.F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, p. 14.11-14.17, 1989). Other methods for detecting the MS1206 specific sequence include the use of allele-specific oligonucleotides during PCR, the oligonucleotide ligation assay, or ligase chain reaction. The latter three methods can be designed to target the individual nucleotides that differentiate MS viruses from other HHV-6 subtypes during the amplification process (White, T.J., Amplification Product Detection Methods. In *Diagnostic Molecular Microbiology: Principles and Applications*, Persing, D.H., Smith, T.F., Tenover, F.C., and White, T.J., eds. Mayo Foundation, pp. 138-148, 1993).

Example 3. Further Characterization of MSV-1206

Summary:

We conducted a phylogenetic characterization and prevalence study of HHV-6 viral subtypes in control and multiple sclerosis patients to further determine the characteristics of virus MSV-1206. We developed a PCR-based assay for HHV-6 that uses a two stage amplification with nested primer pairs to detect less than 10 viral copies in 1 ug of DNA template. We then investigated multiple sites along the viral genome to search for polymorphic markers that could be used to characterize differences between the MS strains and those found in normal individuals. With this more sensitive assay, we have demonstrated that greater than 80% of

individuals contain HHV-6 Type B Group 2 viruses, which differ qualitatively by signature nucleotide alterations from the viruses that are most prevalent in the literature, Z29 (Type B Group 1 virus) and U1102 (Type A virus). More importantly, among the Type B Group 2 viruses, these viruses can be further subtyped into a class that is highly associated with multiple sclerosis, and a separate class that is found in individuals without MS.

MSV-1206 is therefore a member of a subset of the HHV-6B Group 2 subfamily of HHV-6 viruses, and has linked markers that can be used to characterize this virus and its closest relatives. The markers and their location on the MSV-1206 genome is shown in Figure 2 and Table 1. In addition to these qualitative changes, the MS viruses appear to be in higher titers in patients with MS.

Molecular Phylogeny of MSV-1206

The HHV-6 family comprises a large genome and it is impractical and unnecessary to sequence the entire genome of each viral isolate or variant. We focused on nucleotide sequences that may be conserved in viruses in MS patients and that are not conserved in normal patients. Since complete nucleotide sequences for the HHV-6 viruses are not published, we initially analyzed phylogenetically informative regions within virus MSV-1206 that have been previously used to differentiate between HHV-6 Types A or B and Types B1 or B2. This data demonstrated that the MSV-1206 virus belonged to the Type B2 class. We then developed markers that would differentiate among Type B2 viruses. Prevalence data at this level does not exist in the literature, and therefore it was desirable to determine the overall prevalence of viral subtypes in controls as well as in our MS cases.

The map of the HHV-6 genome including regions analyzed is shown in Figures 1 and 2. Phylogenetic analysis of the pZVH14 locus and variable glycoprotein region (vg), which allows differentiation of Type A versus Type B viruses demonstrated that the MSV-1206 virus belonged to the Type B Group of HHV-6. Analysis of the immediate early region (IE) and glycoprotein H region (gH) demonstrated that MSV-1206 is

most homologous to the group 2 branch (isolates H622, H623 from Chou, S. and Marousek, G., 1994, *Virology* 198:370-376; Gompels, *et al.*, 1993, *J. Gen. Virol.* 74:613-622, both incorporated by reference herein and Yeo, *et al.*; Martin, *et al.*; and Josephs, *et al.*, all *supra*) of the HHV-6B class of viruses (whereas the Z29 strain, prototype B, trees to the group 1 branch). A total of 17/17 MS cases and 30/31 isolates from normal patients also fell into the group 2 branch, demonstrating that this was the most prevalent viral type found in our MS population.

In order to classify MSV-1206 as a Type A versus Type B strain, five independent regions of the MSV-1206 virus were amplified and characterized with respect to their sequence similarity to corresponding regions of HHV-6A and HHV-6B strains, including the immediate early region, the DNA polymerase region, a *Hind*III restriction fragment length polymorphism within a *Bam*HI fragment, the major DNA binding protein region (described above as the segment identified by RDA, and containing the characteristic *Hind*III restriction site), and a segment of a variable glycoprotein gene. Although DNA analysis confirmed that MSV-1206 is within the HHV-6 family, qualitative differences were found between the virus isolated from patient MSV-1206 and the prototype strains for HHV-6A (U1102) and HHV-6B (Z29). MSV-1206 differed from the most prevalent HHV-6B virus reported in the literature (strain Z29) in three of these regions, including nucleotide differences in the immediate early region, the major DNA binding protein, and the *Hind*III site within a *Bam*HI fragment that is used to type differences between HHV-6A and HHV6-B strains. In all three of these regions, the MSV-1206 virus does not type as Z29, which is the prototype strain for the class of HHV-6B viruses that cause roseola.

Finally we completely sequenced the Z29 and MSV-1206 phosphotransferase genes and the DNA polymerases of Z29 and MSV-1206.

The following is a summary of the molecular details of each loci that was examined in MSV-1206 and related viruses:

1. Major DNA Binding Protein

The most diagnostic region that differed between the published Z29 sequence and MSV-1206 is the *Hind*III site within the major DNA binding protein (MDBP) identified in MSV-1206 by RDA. We constructed PCR primers that flanked this site. Reference may be made to bases 1141 - 2880 from GenBank record HH6KAHC. This is the portion of the major DNA binding protein gene from strain Z29 that includes the homologue of the *Hind*III fragment that we isolated by RDA from the MSV-1206 virus. The *Hind*III sites that define the fragment are AAGCTT and AAGCTC. Note that the second site at position 1810 is AAGCTT in MSV-1206, and therefore a cleavage site for *Hind*III, while the corresponding sequence in Z29 is AAGCTC, and therefore cannot be cut by *Hind*III. We synthesized the following primers to amplify fragments that include this polymorphic *Hind*III site:

HHV-1	5'-CTATCCCTCATCACCTCAGC-3'	Seq. ID. No. 13
HHV-3	5'-TGAGAACCTTGCCCTTGACC-3'	Seq. ID. No. 50
MSHin9	5'-TAATTGTCTCACGCCGAACC-3'	Seq. ID. No. 15
HHV-16	5'-CTGTGATTGCGTCACTGTCC-3'	Seq. ID. No. 16
HHV-6	5'-GTGGTGTTAGACAAGCAGCC-3'	Seq. ID. No. 14

Initial amplifications were done with either the HHV-1/HHV-6 or the HHV-3/HHV-6 primer pair, using up to 1 microgram of template DNA. Secondary amplifications were done with the nested primer pair MSHind9/HHV-16. The resulting PCR products were then subjected to *Hind*III digestion. This site is lacking in the reported Z29 strain. This site was not previously reported as present in the group 2 branch of HHV-6, or in the viruses we detected in our control population, so a prevalence study was performed for this informative site. We constructed PCR primers that flanked this site and upon restriction endonuclease digestion, found that 37% (10/27) MS cases and 7.6% (2/26) of non-MS controls were positive for the presence of this site. These results are statistically significant. Thus, MSV-1206 represents a subset of HHV-6 Type B viruses that are associated with MS, and this site represents a marker for this class of viruses.

We sequenced the entire gene encoding the DNA binding protein of MSV-1206 virus and present it as Sequence ID No. 81.

2. Immediate Early Region: A 295bp sequence from the RF4 gene in the immediate early region of HHV-6 was sequenced and compared to previously characterized HHV-6B isolates. This ORF (open reading frame) was first identified for U1102 in Martin *et al.*, 1991, *J. Virol.* 65:5381-5390 incorporated by reference herein (GenBank locus HH6IELOC). The same region has been sequenced for a number of HHV-6B strains (in Chou, S. and Marousek, G., 1993, *Virology* 198:613-622) and is available in the following GenBank loci: HST (HH6IMMEA), Z29 (HH6IEXB), H322 (HH6IEXC), H387 (HH6IEXD), H622 (HH6IEXE), H623 (HH6IEXE).

The following primers were used to amplify and sequence this region from a number of MS and non-MS samples:

H6D21: 5'-TTGAAACTCAACCCGACTC-3' (Sequence ID No. 55)
H6D24: 5'-CAGTTTCATAACCAAATG-3' (Sequence ID No. 56)

Flanking distal primers were necessary in a nested two-stage PCR to obtain products in some cases. A sequence was obtained from eight individuals with MS: MS473, MS536, MS573, MS579, MS1206, MS1721, MS1862, MS1864. All eight individuals contained the following sequence (in the immediate early region listed 5' to 3') designated "8/8MS" in Table 4 below. We also sequenced the same region from six individuals who did not have MS: pblA, DA, pblB, pblF, Stim, A1245. These sequences, as well as the sequences from GenBank mentioned above, are aligned relative to the eight MS sequences:

Table 4. Immediate Early Region (listed 5' to 3')

ATTTTGGCTT	ACAGCCCCGA	TCAAAGGTCA	CAGACAAAAG	AAAGGATTCA	8/8MS*
----------	----------	----------	----------	----------	U1102
		A		T	pblA*
		A		T	DA*
		A		T	Z29
		A		T	H322
		A		T	H387
					pblB*
					pblF*
					Stim*
					A1245*
					HST
					H622
					H623

GGAAAAAGGT	TCTAACTCCA	AGTGTACCGA	AACGCTTCCT	TGTATGACCT	8/8MS*
----------	----------	----------	----------	----------	U1102
				G	pblA*
				G	DA
				G	Z29
				G	H322
				G	H387
					pblB*
					pblF*
					Stim*
					A1245*
					HST
					H622
					H623

TTACCGACTC	AGCAACTCCT	GTCAAAAGTC	ATGATGCAAT	TCAGGATACT	8/8MS*
----------	----------	----------	----------	----------	U1102
A			G		pblA*
A			G		DA
A			G		Z29
A			G		H322
A			G		H387
					pblB*
					pblF*
					Stim*
					A1245*
					HST
					H622
					H623

TTAAATCCAG	AAAGTAAAAT	AGACAAAGAA	TTGGAAGCTG	TAGAAAGTCT	8/8MS*
----------	----------	----------	----------	----------	U1102
C	C T		A	G	pblA*
C	C T		A	G	DA*
C	C T		A	G	Z29
C	C T		A		H322
C	C T		A		H387
					pblB*
					pblF*
					Stim*
					A1245*
			G		HST
					H622
					H623

```
AGTGAATCTA TGTGATGGTT TCCATGACAA CCCTTTAATA TCAGAAATGA 8/8MS*
---------- -----      C A   G                        T U1102
                                                      pb1A*
                                                      DA*
                                                      Z29
                                                      H322
                                                      H387
                                                      pb1B*
                                                      pb1F*
                                                      Stim*
                                                      A1245*
                                                      HST
                                                      H622
                                                      H623

TAACATTTGG TTATGAAACT GATCACTCAG CACCTTATGA GAGTGA 8/8MS*
 G TG                          G      C             U1102
                                                    pb1A*
                                                    DA*
                                                    Z29
                                                    H322
                                                    H387
                                                    pb1B*
                                                    pb1F*
                                                    Stim*
                                                    A1245*
                                                    HST
                                                    H622
                                                    H623
```

In the above Table 4, only differences relative to the MS sequence are shown. A dash indicates that a base present in the MS sequence is absent in the U1102 (Type A HHV-6) sequence. An asterisk indicates that a sequence was obtained by us. Sequences 8/8MS, U1102, pb1A, DA, Z29, H322, H387, and HST correspond to Sequence ID Nos. 57 to 64, respectively. (Sequences pb1F*, Stim*, A1245*, H622 and H623 are all identical to pb1B* and 8/8MS and correspond to Sequence ID No. 57.)

3. DNA Polymerase sequence comparison: We have completely sequenced the DNA polymerase gene of MSV-1206 virus and present it Seq. ID. No. 82. The complete sequence for the polymerase gene for HHV-6A strain U1102 (4407bp) is GenBank record HH6DNAPOL. Partial 466bp sequences for strain AJ (Type B) is GenBank record HH6DNAPOL2. Both are referenced in Teo, I.A., *et al.*, 1991, *J. Virol.* 65:4670-4680.

4. *Hind*III RFLP within the *Bam*HI fragment: A *Hind*III RFLP has been extensively used to differentiate between HHV-6 subtypes A and B. This site was first noted in Aubin, J.T., *et al.*, 1991, *J. Clin. Micro.* 29:367-372, where it was shown that subtype A strains HBLV, TAN, and SIE do not have the site, while subtype B strains HST, BOU, MAR, BLE, and MBE have the site. Although the genes located in this region have not been defined, the RFLP has been mapped to an 850 bp *Bam*HI fragment that is contained within the *Hind*III H fragment of U1102 originally subcloned in pZVH14 (see Josephs, *et al.* 1986, *Science* 234:601-603. The *J. Clin. Micro.* paper reports short sequences from strains SIE (subtype A) and HST (subtype B) that show the *Hind*III site AAGCTT in subtype B is replaced by AAACTT in subtype A:

```
TCTGTTCCAG AGAAAGGGTG TTGCGAAGGG CTGATTAGGA TTAATAGGAG SIE
                                              C  G     HST

AATCTTGTAA GTATATTGGT CCAGTTGAAA TTAGATTTCA TTATAGGAAA SIE
                                 T  G                  HST

AGATTTGAGA TTCGTGAAAT AAAGATTGAA ATTGTGATCG TAGTTCATAA SIE
                                                       HST

AAACTTGGAA TGAGG SIE (Sequence ID No. 65)
  G         C    HST (Sequence ID No. 66)
```

Two primers are reported in the *J. Clin. Micro.* paper, *supra*, that amplify a 249 bp fragment with the *Hind*III RFLP located approximately 30 bp from one end of the fragment. The primers are:

primer A 5'-GATCCGACGCCTACAAACAC-3' (Sequence ID No. 67)
primer B 5'-TACCGACATCCTTGACATATTAC-3' (Sequence ID No. 68)

We used these primers to amplify the 249 bp fragment from MSV-1206, demonstrating that this locus is present in the MSV-1206 virus. We found that the MSV-1206 249 bp fragment can be digested to a 220 bp and a 30 bp fragment by *Hind*III. Thus, the *Hind*III site that is present in all HHV subtype B strains that have been analyzed to date is also present in the MSV-1206 virus.

5. Glycoprotein Gene: The protein coding sequence of glycoprotein gene "BHLF2" from strain U1102 has been reported in Gompels, *et al.*, *DNA seq.* 3:25-39 (1992) (1943-3898 in GenBank record HV6IDDNA). The homologous glycoprotein gene "KA8LA" from strain Z29 can be found in GenBank record HH6KAHC, bases 12678-14894. The alignment of bases 13328-13941 from Z29 with bases 2413-2858 from U1102 shown below reveals two regions with insertions in the Z29 sequence relative to the U1102 sequence that can be used in a PCR-based assay to distinguish these two strains:

```
       primer 1>                                    primer 2>
TTGGAGTGAC AGACAACGTC TGAACGGTAG GTGGCTCGCT TGAGGATTTC Z29
                            T        A     T           U1102

AAAGTTGTCC TCTGGAATCT GTCCTCTTTG AAGGTTTTGA CGGTCTGCAC Z29
  G                   T     ------- ---------- ---------- U1102

 TTCTGTGCTA TGGGTTTCGT TTGCGTATTC TGTTTGGTTT TTTACGTTGA Z29
---------- ---------- ---------- ---------- ---------- U1102

                <primer 4
GAAGTTGGGT AGGATTTGTG GGTTCTTTAG GCGTGATTCC CATCGTTTCT Z29
---------- ---------- ---------- ---------- ---------- U1102

                                          <primer 5
CCAAGAGAAA TTTTAATAAA ATTTTCGGTC ATGTTTTGTA CTTCCGTGTT Z29
---------- ---------- ------                       T    U1102

                                                <primer 6
GTGGCTCATG TATGTGCGCG CCGCGTCTTG CCAAGTGGTA AACTTTAAAA Z29
   A                TA    T  T                         U1102

TCGGAGTGGT GTGTTCGGTG TCGGTGAATT TTGTCGTGCT CTGCTGTGAT Z29
                    C                  T  A             U1102

TTGCTCTTAA GATAAATAGA GTTTTTTGGT GAAGAATAGA TATTGTCGCT Z29
                                           G      C  T  U1102

AATTTCCTCG ATTCCTATTG TTGTTGCGAA GGTGGTGCTT TCGAGCTTTT Z29
        T                  ---                    T    U1102

GAGTCGTGTT TGTTGCCACT GGTAGTGCTC TCAAACGTTT CGGGAATTCT Z29
 G  T C         TTG   -    C         GG     T           U1102

 primer 11>              primer 12>
GATTGTGGTA TTTTCAAAAT TTGTTGGTTT TGGTGGACTT TTTGGGTTTT Z29
                                                       U1102

                                  <primer 13
CGGTAGGGTT TTCGGTGTTT TGTTCGGTTT TAATAGTGGC GTTACGACGG Z29
   ------- ------                                 A    U1102

 <primer 14
ATTGTAAGAG TTGGA Z29    (Sequence ID No. 69)
                U1102  (Sequence ID No. 85)
```

(Sequence ID Nos. corresponding to primers 1, 2, 4-6, 11-14 above are 70-78, respectively.)

Depending on whether the viral template is more closely related to the Z29 or the U1102 strain, the expected sizes of the PCR products produced with specific pairs of the primers will be:

PRIMER PAIR	U1102	Z29
1/6	145	298
2/5	62	215
2/4	0	145
11/14	110	122
12/13	62	74

We used these five primer pairs to amplify products from the MSV-1206 virus and found that the sizes are identical to those predicted for Z29. This shows that this region is present in MSV-1206 and that the virus strain is more closely related to the subtype B than to the subtype A class of HHV-6 viruses.

With further analysis, we have sequenced the entire glycoprotein gene of the MSV-1206 virus and present it as sequence ID No. 83.

6. The phosphotransferase gene of MSV-1206.

We have completely sequenced the phosphotransferase gene from MSV-1206 and compared it to the corresponding gene in Z29. The two genes are presented in Sequence ID Nos. 84 and 79, respectively.

Example 4. HHV-6 Immunofluorescence Assay

The following protocol is useful for screening for potential MS drugs. Human umbilical cord blood cells (5 x 10^5 cells) are mixed with a 50% TCID (tissue culture infection dose as measured by cytopathic effects after 5-6 days) and spun at 1800 rpm for 10 min. The inoculated cells are incubated at 37°C for 30 min. before transferring them to 12 well plates in 2 ml of cord blood media (+/- drug). After incubation in a CO_2 incubator for 5-12 days, aliquots of the cells are examined for cytopathic effects. To quantitate the effect on the drug on replication, aliquots of cells are fixed onto slides and stained with anti-HHV-6 human positive control serum and anti-human Ig-FITC with Evan's Blue as a counter stain. The total cell count and stained cell count is

determined by examination under a fluorescence microscope. The percentage of bright cells relative to the zero drug control are plotted vs. drug concentration on a semi-log plot. The IC50 is reported as the concentration of drug in micrograms per milliliter that inhibits the staining pattern by 50%. Following the above protocol we obtained the following results.

Compound	HHV-6A	HHV-6B
Ganciclovir	2	6
Acyclovir	7	50
Foscarnet	17	80
Beta-interferon	6	300
Alpha-interferon	40	2,000

Example 5. Detection of HHV-6 in brain tissue.

HHV-6 was localized in brain tissues by immunocytochemistry (ICC) with antibodies to viral antigens and by *in situ* hybridization with DNA probes encompassing 8 kb of the genome. For immunocytochemistry, murine monoclonal antibodies to antigens p101 and p41/38 were used as the primary antibody (Chemicon Corp.), followed by a biotinylated goat anti-mouse secondary antibody. The detection system consists of a streptavidin-horseradish peroxidase conjugate with diaminobenzidine as the colorimetric marker (BioGenex Corp.). The *in situ* hybridization probe is 8 kilobases of PCR amplified MS-1 viral DNA, in which digoxigenin is incorporated during the PCR reaction. The detection system consisted of a sheep anti-digoxigenin Fab fragment conjugated to alkaline phosphatase. The colorimetric marker is nitroblue tetrazolium/bromochloroindole phosphate (NBT/BCIP, Boehringer Mannheim Corp.)

In control brain samples, HHV-6 was primarily localized in microglia and astrocytes of the subpial and subependymal regions, with patchy low level staining of the overlying ependymal cells and meningoepithelial cells. The cortex and white matter from normal brains showed sporadic low level staining of neurons (10-20%) and glial cells (<1/1000).

In MS cases, particularly those with active inflammation or plaques, both the intensity of staining and percentage of positive neurons and glial cells were greater than in controls (Table 5). The regions of gray matter surrounding both inactive and active plaques contained a high percentage (>90%) of stained neurons. In the white matter, in areas of demyelination, positively astrocytes and oligodendroglia were observed in and around plaques (3-4% of oligodendroglia and astrocytes). The intensity of staining was patchy in MS cases; the histologically uninvolved regions of MS brains showed a pattern of HHV-6 staining similar to that seen in controls. Areas of intense staining (95% neurons, 20% oligodendrocytes) were observed in a case without evidence of inflammation or demyelination, strongly suggesting that viral activation can be antecedent to the inflammation. We conclude that HHV-6 is neurotropic and is associated or causative of the lesions that are characteristic of MS.

Table 5. Summary of Mapping MS Cases with p101

Case	Neurons	Oligos
MS-1	95%	3%
MS-2	95%	4%
MS-3	90%	2%
MS-4	95%	3%
MS-5	95%	1%
MS-6	90%	3%
MS-7	95%	4%
MS-8	95%	3%
MS-4*	95%	20%

*Focus of HHV-6 activation without plaque

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were

specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: PathoGenesis Corporation
(B) STREET: 201 Elliott Avenue W., Suite 150
(C) CITY: Seattle
(D) STATE: Washington
(E) COUNTRY: U.S.A.
(F) POSTAL CODE (ZIP): 98119
(G) TELEPHONE: (206) 467-8100
(H) TELEFAX: (206) 282-5065
(I) TELEX:

(ii) TITLE OF INVENTION: VIRAL ASSOCIATED MULTIPLE SCLEROSIS: TREATMENTS, PREVENTION AND DIAGNOSIS THEREOF

(iii) NUMBER OF SEQUENCES: 85

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(v) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: WO not yet assigned
(B) FILING DATE: 04-NOV-1994
(C) CLASSIFICATION:

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/287,942
(B) FILING DATE: 05-MAR-1994

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/218,029
(B) FILING DATE: 24-MAR-1994

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/149,176
(B) FILING DATE: 05-NOV-1993

(vii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Weber, Kenneth A.
(B) REGISTRATION NUMBER: 31,677
(C) REFERENCE/DOCKET NUMBER: 15371-4-3PC

(viii) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (415) 543-9600
(B) TELEFAX: (415) 543-5043

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGAAAAGACG TTGGACAATC 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AAGAGACAGT CGATGTGATC 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACGTTCACGA ACCTCGATA 19

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AGATGGCTCT CTGTTGTTC 19

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACTGTCATCC AGCAGGTATA GAAACGAGAA GCTCATGCTT 40

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ACTGTCATCC AGCAGGTATC GAAACGAGAA GCTCATGCTT 40

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGGAGATGTT TGGTAGTTC 19

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAAACACTTC CATGAATTCG 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGGAACGATG CCTTC 15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GAAGATATTG AACGAGA 17

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTAATTTTAT TGACAAACTC GGGGAAACTC CCGCACTTCC 40

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTAATTTTAT TGACAAACTT GGGGAAACTC CCGCACTTCC 40

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTATCCCTCA TCACCTCAGC 20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTGGTGTTAG ACAAGCAGCC 20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TAATTGTCTC ACGCCGAACC 20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGTGATTGC GTCACTGTCC 20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TATTCCCTAT TAGCCAAGCT TACAAATTTC TCTAATGTCA A 41

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TATTCCCTAT TAGCCAAGCT CACAAATTTC TCTAATGTCA A 41

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGGTACGACT TGAGACG 17

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CCATCGACTT CAAACACC 18

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CTGAAAATGA TCCTTCGTG 19

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

ATCTTTACCG AACCATGATC 20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GCTGTTAAAT TCACGTTAAC AGAGCAAGTT CTTCGAGGT 39

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GCTGTTAAAT TCACGTTAAA AGAGCAAGTT CTTCGAGGT 39

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TAGAATTTCA ATATTTAATT TCACATGTAA TTTAAGCATT T 41

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TAGAATTTCA ATATTTAATT CCACATGTAA TTTAAGCATT T 41

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCTATGCGTG ATGGAACG 18

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TCTCATCTTG TGGAGCAC 18

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

ACGATCATGT TGTGAGCG 18

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AAATGCCAGT CCAACAGC 18

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TGCTTTAATC TCCGGAAATG TTGTGATATG GCGCGATTTA G 41

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TGCTTTAATC TCCGGAAATG CTGTGATATG GCGCGATTTA G 41

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TGTTGTCTGT TCAGGCAGCT GAAATCCGAA ACTCTTCCAA A 41

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TGTTGTCTGT TCAGGCAGCT AAAATCCGAA ACTCTTCCAA A 41

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TATGGCCTAT TGCATGGC 18

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

AAACACCTAT GGTTCGCC 18

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CATGTGATGA TCACACCG 18

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

AATCAGCAAA GAGCCGAG 18

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GAAATGTTTC NCGTGTACGC GGCGCGAAAA GGGGTCGGAT T 41

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GAAATGTTTC NCGTGTACGC AGCGCGAAAA GGGGTCGGAT T 41

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CTGCAGGATA ACCCAGAGC 19

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CTTCATCCTG AACGTGATCT 20

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

TACGCACATT GTTTGCGC 18

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

ATCCACTGTA GTAGGGC 17

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

AGGCATCACA AAAATTATCA GATTCCCTTT TAACTCTTTG ACTCTC 46

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

AGGCATCACA AAAATTATCA GGTTCCCTTT TAACTCTTTG ACTCTC 46

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 420 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CAGTTTAAGC ATTATGGATG CCGCTAAAGC TTCCCTACCA TCCACAAAAA AAAGCATATC 60

ATCCATAGTC GGCTTGTCCT CCCTATCCCT CATCACCTCA GCTATTATTA ATAATTCTGG 120

ATCAATATCG TTAGTTAAAT TCTCAACAAT GCTGAGAACC TTGCCCTTGA CCACTTCCGT 180

ATCAAACATA ATTGTCTCAC GCCGAACCTT TTTCACTATA ACTTCTGAGA ACTTTGTAGC 240

AATAATCGTT TTCTGCCTCA TAAATCTAAA ATCTTGCAAT GCAGAAGAGG TAGGGTTTAA 300

GTTCCTATCC ACGCCACTTC CACCTATCCA ACCTAACTGG CCAAACTGAA AATATTCCCT 360

ATTAGCCAAG CTCACAAATT TCTCTAATGT CAAACCAAAT GTAACCAAAG ATCGACTTGT 420

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 420 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

CAGTTTAAGC ATTATGGATG CCGCTAAAGC TTCCCTACCA TCCACAAAAA AAAGCATATC 60

ATCCATAGTC GGCTTGTCCT CCCTATCCCT CATCACCTCA GCTATTATTA ATAATTCTGG 120

ATCAATATCG TTAGTTAAAT TTTCAACAAT GCTGAGAACC TTGCCCTTGA CCACTTCCGT 180

ATCAAACATA ATTGTCTCAC GTCGAACCTT TTTCACTATA ACTTCTGAGA ACTTTGTAGC 240

AATAATCGTT TTCTGCCTCA TAAATCTAAA ATCTTGCAAT GCAGAAGAGG TAGGGTTTAA 300

GTTCCTATCC ACGCCACTTC CACCTATCCA ACCTAACTGG CCAAACTGAA AATATTCCCT 360

ATTAGCCAAG CTTACAAATT TCTCTAATGT CAAACCAAAT GTAACCAAAG ATCGACTTGT 420

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GGCCAGTTAG GTTGGATAGG 20

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

TGAGAACCTT GCCCTTGACC 20

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

TGGTCAAGGG CAAGGTTCTC 20

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

ATGCAACATC AGCGGATG 18

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GTGAAAGAAC GAAGGACG 18

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

TACGAGACTA GGATAGCG 18

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

TTGAAACTCA ACCCGACTC 19

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CAGTTTCATA ACCAAATG 18

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 296 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

ATTTTGGCTT ACAGCCCCGA TCAAAGGTCA CAGACAAAAG AAAGGATTCA GGAAAAAGGT 60

TCTAACTCCA AGTGTACCGA AACGCTTCCT TGTATGACCT TTACCGACTC AGCAACTCCT 120

GTCAAAAGTC ATGATGCAAT TCAGGATACT TTAAATCCAG AAAGTAAAAT AGACAAAGAA 180

TTGGAAGCTG TAGAAAGTCT AGTGAATCTA TGTGATGGTT TCCATGACAA CCCTTTAATA 240

TCAGAAATGA TAACATTTGG TTATGAAACT GATCACTCAG CACCTTATGA GAGTGA 296

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

TGGTTCCAAT GGCAACCCTT TAATATCAGA AATGATGATG TTTGGTTATG AAACTGATCA 60

CTCGGCACCC TATGAGAGTG A 81

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 296 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

ATTTTGGCTT ACAGCCCCGA TAAAAGGTCA CAGACAAAAG AATGGATTCA GGAAAAAGGT 60

TCTAACTCCA AGTGTACCGA AACGCTTCCT GGTATGACCT TTACCAACTC AGCAACTCCT 120

GTCAAAAGTC ATGGTGCAAT TCAGGATACT CTAAATCCAG AAAGCAAATT AGACAAAGAA 180

ATGGAAGCTG TAGAGAGTCT AGTGAATCTA TGTGATGGTT TCCATGACAA CCCTTTAATA 240

TCAGAAATGA TAACATTTGG TTATGAAACT GATCACTCAG CACCTTATGA GAGTGA 296

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 296 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

ATTTTGGCTT ACAGCCCCGA TAAAAGGTCA CAGACAAAAG AATGGATTCA GGAAAAAGGT 60

TCTAACTCCA AGTGTACCGA AACGCTTCCT GGTATGACCT TTACCAACTC AGCAACTCCT 120

GTCAAAAGTC ATGGTGCAAT TCAGGATACT CTAAATCCAG AAAGCAAATT AGACAAAGAA 180

ATGGAAGCTG TAGAGAGTCT AGTGAATCTA TGTGATGGTT TCCATGACAA CCCTTTAATA 240

TCAGAAATGA TAACATTTGG TTATGAAACT GATCACTCAG CACCTTATGA GAGTGA 296

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 296 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

ATTTTGGCTT ACAGCCCCGA TAAAAGGTCA CAGACAAAAG AATGGATTCA GGAAAAAGGT 60

TCTAACTCCA AGTGTACCGA AACGCTTCCT GGTATGACCT TTACCAACTC AGCAACTCCT 120

GTCAAAAGTC ATGGTGCAAT TCAGGATACT CTAAATCCAG AAAGCAAATT AGACAAAGAA 180

ATGGAAGCTG TAGAGAGTCT AGTGAATCTA TGTGATGGTT TCCATGACAA CCCTTTAATA 240

TCAGAAATGA TAACATTTGG TTATGAAACT GATCACTCAG CACCTTATGA GAGTGA 296

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 296 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

ATTTTGGCTT ACAGCCCCGA TAAAAGGTCA CAGACAAAAG AATGGATTCA GGAAAAAGGT 60

TCTAACTCCA AGTGTACCGA AACGCTTCCT GGTATGACCT TTACCAACTC AGCAACTCCT 120

GTCAAAAGTC ATGGTGCAAT TCAGGATACT CTAAATCCAG AAAGCAAATT AGACAAAGAA 180

ATGGAAGCTG TAGAAAGTCT AGTGAATCTA TGTGATGGTT TCCATGACAA CCCTTTAATA 240

TCAGAAATGA TAACATTTGG TTATGAAACT GATCACTCAG CACCTTATGA GAGTGA 296

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 296 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

ATTTTGGCTT ACAGCCCCGA TAAAAGGTCA CAGACAAAAG AATGGATTCA GGAAAAAGGT 60

TCTAACTCCA AGTGTACCGA AACGCTTCCT GGTATGACCT TTACCAACTC AGCAACTCCT 120

GTCAAAAGTC ATGGTGCAAT TCAGGATACT CTAAATCCAG AAAGCAAATT AGACAAAGAA 180

ATGGAAGCTG TAGAAAGTCT AGTGAATCTA TGTGATGGTT TCCATGACAA CCCTTTAATA 240

TCAGAAATGA TAACATTTGG TTATGAAACT GATCACTCAG CACCTTATGA GAGTGA 296

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 296 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

ATTTTGGCTT ACAGCCCCGA TCAAAGGTCA CAGACAAAAG AAAGGATTCA GGAAAAAGGT 60

TCTAACTCCA AGTGTACCGA AACGCTTCCT TGTATGACCT TTACCGACTC AGCAACTCCT 120

GTCAAAAGTC ATGATGCAAT TCAGGATACT TTAAATCCAG AAAGTAAAAT AGACAAAGAA 180

TTGGGAGCTG TAGAAAGTCT AGTGAATCTA TGTGATGGTT TCCATGACAA CCCTTTAATA 240

TCAGAAATGA TAACATTTGG TTATGAAACT GATCACTCAG CACCTTATGA GAGTGA 296

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

TCTGTTCCAG AGAAAGGGTG TTGCGAAGGG CTGATTAGGA TTAATAGGAG AATCTTGTAA 60

GTATATTGGT CCAGTTGAAA TTAGATTTCA TTATAGGAAA AGATTTGAGA TTCGTGAAAT 120

AAAGATTGAA ATTGTGATCG TAGTTCATAA AAACTTGGAA TGAGG 165

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

TCTGTTCCAG AGAAAGGGTG TTGCGAAGGG CTGATTAGGA TTCATGGGAG AATCTTGTAA 60

GTATATTGGT CCAGTTGAAT TGAGATTTCA TTATAGGAAA AGATTTGAGA TTCGTGAAAT 120

AAAGATTGAA ATTGTGATCG TAGTTCATAA AAGCTTGGAA TCAGG 165

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

GATCCGACGC CTACAAACAC 20

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TACCGACATC CTTGACATAT TAC 23

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 615 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

TTGGAGTGAC AGACAACGTC TGAACGGTAG GTGGCTCGCT TGAGGATTTC AAAGTTGTCC 60

TCTGGAATCT GTCCTCTTTG AAGGTTTTGA CGGTCTGCAC TTCTGTGCTA TGGGTTTCGT 120

TTGCGTATTC TGTTTGGTTT TTTACGTTGA GAAGTTGGGT AGGATTTGTG GGTTCTTTAG 180

GCGTGATTCC CATCGTTTCT CCAAGAGAAA TTTTAATAAA ATTTTCGGTC ATGTTTTGTA 240

CTTCCGTGTT GTGGCTCATG TATGTGCGCG CCGCGTCTTG CCAAGTGGTA AACTTTAAAA 300

TCGGAGTGGT GTGTTCGGTG TCGGTGAATT TTGTCGTGCT CTGCTGTGAT TTGCTCTTAA 360

GATAAATAGA GTTTTTTGGT GAAGAATAGA TATTGTCGCT AATTTCCTCG ATTCCTATTG 420

TTGTTGCGAA GGTGGTGCTT TCGAGCTTTT GAGTCGTGTT TGTTGCCACT GGTAGTGCTC 480

TCAAACGTTT CGGGAATTCT GATTGTGGTA TTTTCAAAAT TTGTTGGTTT TGGTGGACTT 540

TTTGGGTTTT CGGTAGGGTT TTCGGTGTTT TGTTCGGTTT TAATAGTGGC GTTACGACGG 600

ATTGTAAGAG TTGGA 615

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GGAGTGACAG ACAACGTC 18

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GGCTCGCTTG AGGATTTC 18

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GGGTAGGATT TGTGGGTTCT T 21

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GGTCATGTTT TGTACTTCCG T 21

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

GCCAAGTGGT AAACTTTAAA A 21

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GAATTCTGAT TGTGGTATTT TC 22

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

AATTTGTTGG TTTTGGTGGA C 21

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

GTTCGGTTTT AATAGTGGCG 20

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

GACGGATTGT AAGAGTTGGA 20

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1692 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

ATGGACAACG GTGTGGAGAC ACCTCAAGGT CAAAAAACTC AGCCGATAAA TTTGCCACCA 60

GACAGGAAAA GGTTGAGAAA ACATGACGGA CTTGGAAAAG GTGTTAAACG AAAACTTTTT 120

GCCGAAGATA GCTCTCCGTT AAAGAAACAG ATCCCCGCCT GCAGCGATAT GGAAACACTT 180

TCTTCGCCTG TAAAGTTTGG ATGCAAGTCG CGAAGTGCTT CTGCTCTCGA TGAAAGTTTC 240

GGAAAATGTA AACACGAAAC TGCTTGCGAT TGTTCTGCGA TAGAGGAATT GCTTTGTCAC 300

GAGTCGCTTT TAGACTCGCC GATGAAACTG TCGAATGCCC ACACCATCTT CAGCTCAGAC 360

AAATGGAAAC TGGAGCTAGA GAAAATTATA GCTTCAAAGC AGATCTTTCT AGACATGAGC 420

GAGAATGTTG AACTTGTAGC CTACGGCGAG ACTTTGTGTA ACCTGAGAAT TTACGAAAAG 480

ATCAGCTCGC CGTTTTTGTT TGACGTGCAA AGCGAGGAGC GTTCGTATTC AGTGGTTTAC 540

GTCCCTCACA ACAAAGAACT TTGTGGACAG TTTTGTCAAC CTGAAAAGAC TATGGCTCGA 600

GTTCTCGGAG TGGGTGCCTA CGGGAAGGTG TTTGATCTAG ATAAAGTGGC CATAAAGACG 660

GCCAACGAAG ACGAGAGTGT CATTTCGGCT TTTATAGCCG GTGTCATCCG TGCAAAATCG 720

GGAGCCGACT TATTATCTCA CGACTGTGTT ATTAATAACC TTCTGATTTC AAATTCCGTT 780

TGTATGGATC ATAAAGTGTC TTTGTCACGT ACTTATGATG TTGATCTCTA TAAGTTCGAA 840

GATTGGGATG TCAGGAATGT AATGAATTAT TACAGTGTGT TTTGTAAGTT AGCTGATGCT 900

GTAAGGTTTC TAAATCTGAA ATGTAGAATT AATCATTTCG ATATCTCACC TATGAATATC 960

TTTATAAATC ATAAAAAAGA GATAATCTTT GATGCCGTGT TGGCGGATTA CAGCTTGTCC 1020

GAAATACATC CCGAGTATAA CGGCACGTGT GCTATTGCTA AAGAGTATGA CAGAAATCTT 1080

CAACTTGTGC CAATCAGTCG TAACAAATTC TGTGACATGT TTAATCCTGG ATTTCGACCA 1140

CTTGTCGCCA ATGCAATGAT ATTGGTCAAT GTATGCGAGG CTTTTGATGG TGAAAATAAT 1200

CCTCTTAGAC ACTGTAATTT GGATCTGTGC GCCTTTGCTC AGGTCGTATT ATTGTGTGTC 1260

CTGAGAATGA CAGATAAACG CGGATGTCGC GAAGCTCAGC TATACTACGA GAAAAGGTTG 1320

TTTGCGTTGG CTAACGAGGC CTGTCGATTG AATCCTCTTA GATATCCATT TGCTTACAGG 1380

GACGCTTGCT GTAAAGTATT GGCTGAGCAT GTAGTGTTGC TAGGGTTATT GTTTTACCGA 1440

GACGTGGTTG ATATATATGA AAAAATATAC GATTTTCTAG ATGAAAGAGG GGAATTTGGG 1500

TTACGAGACC TGTTTGAGGC AACTTTTTTA AATAATAGTA AACTTACCAG ACGTCAGCCA 1560

ATCGGAGGAG GTCTTGCGTC TCTACAGTCG TCCGAGTATG GAGAAAAACT TTTACATGAC 1620

CTTAGAGCGT TGTTCTTGAT CACTTCTTCT GCAGATCTGG ATAAAGATAC ATCATCTCTC 1680

TTTCAGATGT GA 1692

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

TTTGCTGTTG GACTGGC 17

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3399 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..3399
(D) OTHER INFORMATION: /standard_name= "DNA Binding Protein gene sequence from MS1206 virus"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

ATGGCTGATG AAAACGAAAC GGTGGTTTCT GCTCCTGTTT CTACTGCTGC TTGGATCTAT 60

GTTTTTCCAA AAGAGAAAGA ACTTTTGGAT GTTTTATCTG TTTTATCGTT AATGGAGAGA 120

AATTCTCCTG TAGTTATTTC GCCGTTGTTA ATGAATTTGA CTGTAGAAAA CGATTTCTCG 180

ACCACGGTTA AAACACCTAT CACTAACTTT GGAGGCACAA TTCTAACTAA AATTACGTCT 240

TTTATGCCCG TGTGTTTTTT TTTCCATGGA ACTGAGCAGT TAGTTGGTAT GGCCGAAGAT 300

CATGGTGATC TTATTCGATT ATGTGAGCAA ACCAGACAGA AATTTCACTT GCAGTCGTTT 360

GAGGTGCCTA CAGCACGCAA AGTAATCGAT ATCAAAGCTC TTTGTTCTGC TGTCGGCAAA 420

GATGCAGATT CTGTTATTTG CCATGTTGCG TGTGGAAACG GTTTCAAAGA GTTGTTGTTC 480

GCTGGTCTGT TAATTCCTTG TGTTGAAGAA CAAATTCAAG TACAAGTTGG GGAGTATTCG 540

TGTGTGAAAA TTCCTCTATA CTCTGCAACT TTATTTGAAA CTGAAGAAAC GATTTCTTTA 600

TCTTCCTGTA CAGAATTTAT TCAGGAACGA GGATTTTTTC TGCCTGCGTT AAGTGAAACG 660

CTTTTTTATT ATGTTTTTAC ATCATGGGGT ACAACGTTAC GATTTTCTAA TACTAAGGAA 720

TTAATCGATG CTGGCCTGAA ACAATTTACA CAGGATGGGG AGCAAACTGT CAAATTAGCA 780

CCCCACAAAA CTTATCTGGG TATCTCCGGA CAGAAAATTT CAGCGGTCGA GAAAGATTTT 840

TTGATGTTGG TTGATTCCGT TGTGACAGAA TTATCGTTTA GTCATGTAGC CGAATACCTG 900

GATTCGGTGT ATGACCCCAG TCAGATAATG AATTTCAATG ACTGGCCAAT CATAAGAAAC 960

TCAGAGACAC ACGCTGAACG CATGGCGCAG CTCACGAATT TAAAGTTACA TCTGTCTAGT 1020

CATCTTGCCG TTTTGATCTT TGCGCCAAAT TCTATCTTAT ATTGCTCAAA ATTGGCATTT 1080

ATACCAAACG TGAAACAAGC GTTTAATTCG GTGATGACTC AAGAATTACT GCTGCGATCT 1140

TTGTCTTTTT GTAATGCGTT GTCGTCTTTG ACTGAGGATG TGTATAACGA TAATAGAAAA 1200

ATCATCAAAT GTGATTCGAC ATCAGGCAAA GATGACAAAT TTTCAGCTAA TCACTTGGCT 1260

TATGCCTGTG CGACTTGCCC ACAGCTGCTT TCTTATGTAG TGTGGAATTT AAATCGTATG 1320

AGCGTTTATA ACGCGGGTAA TGCGTACACT GAAATTTACA ATCATCTGGT AAATTGTTCG 1380

GCGAATTTAT GTGAATTCTG TGATGGGAAA TGTTGTCAAT CGTGTATTGG AACTGCCATG 1440

GTGCGGGTTG GTACTCGTTT GCCGGCAATA CCAAAAAATG TTAAAAAGGA ACCACTTGTT 1500

ATGAGCATGT TTTCTCGGTA TTATGCCGAG GTTGATATAT TAGGTTCTTT TGGCAGGAAG 1560

CCTGTCAGCG AGCTCAAAGA GATTGGAAAA GATCAACAAA ACACGCTCTC ACTGGACAGG 1620

GGTAAATTTG TATCTCAGAT CTTCGATTAC TGTAAAAAAA ATTCTCTGAT TGATCCTGTA 1680

ACAGGCGAAG ATACTTTCAA TGTCCGAAGT AAAAAAGATT TTGTTTCTAT AATCCACGGA 1740

TTGACACAAT GTATCGAGGA ATGTGTTTCT AGATGCATTG TTGAAATGAG AAGGACACAA 1800

ACGCCTAGAG AACAGATAGA AAATTGTCTA CAATCATTTA ATGTAGATAC GACCCCTTAT 1860

GCAACTGCAT TTTCTCCTTT TTTGACGTTT GCATATTATA AGGTCATTTT AACGGTTTTG 1920

CAGAACCTCG CATTGATAGT GGCTTCAGGT CATGTTGTCG ACAGACCTTG TACTGGTAAT 1980

TCAATTTCTA AATGGCTCGT TCAGCAGTAT CAATCACTGT ACGGAACGTT TCACAGCAGT 2040

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GAGCAGATCT TAGATTGCGA TCTATATAAA TCGGGTAAAT ATGTGAAAAC TACGATTCAG 2160

GCGAAACTTT GCCGTTTGTC TATGCAATGT CTTCGAGATT TTAGAATAAA AAATAGACCG 2220

TTCAACAAGT CCAGTAAGAC AGCACACAAC AACCCTTATT TTAAAAAGAA CGTGAAACAC 2280

AAGAAGAATC CCTTGTCTGG CTGTATCTCC TTTCTTTTAT TTAAATATCA TGATAAATTG 2340

TTTCCGAATG TCAAAATTTC GTGCTTAGAG TTATGGCAAC GTTTTCTGCT CAACAATGTT 2400

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GGCGTGGATA GGAACTTAAA CCCTACCTCT TCTGCATTGC AAGATTTTAG ATTTATGAGG 2940

CAGAAAACGA TTATTGCTAC AAAGTTCTCA GAAGTTATAG TGAAAAAGGT TCGGCGTGAG 3000

ACAATTATGT TTGATACGGA AGTGGTCAAG GGCAAGGTTC TCAGCATTGT TGAAAATTTA 3060

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AAGCCGACTA TGGATGATAT GCTTTTTTTT GTGGATGGTA GGGAAGCTTT AGCGGCATCC 3180

ATAATGCTTA AACTGAATCA CTTAGTTGAT ATGAATGTGA GAGATTTTTC AATAGCTAAT 3240

TTGCAGTCGG TATTTGAGGC GGTGTCTTCA AACGATGCTC CGGTCTATGA CTTTTCTGAA 3300

ATTCTAGCGG AGGAGGACGA TCAAGCAAGC GGTGTATTAA AATGTGATGA AACGGAAACC 3360

GAAACGGATG AGCCGATGAC TAAGAAAAAC CGATTATAA 3399

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3039 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..3039
(D) OTHER INFORMATION: /standard_name= "DNA Polymerase gene sequence from MS1206 virus"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

ATGGATTCGG TGTCGTTTTT TAATCCATAT TTGGAAGCGA ATCGCTTAAA GAAAAAAAGC 60

AGATCGAGTT ACATTCGTAT ACTTCCTCGC GGTATAATGC ATGATGGTGC TGCGGGCTTA 120

ATAAAGGATG TTTGTGACTC TGAACCGCGT ATGTTTTATC GAGACCGACA GTATTTACTG 180

AGCAAAGAAA TGACTTGGCC GAGTTTAGAC AGAGTTCGGT CCAAGGATTA TGATCATACG 240

AGGATGAAGT TCCACATCTA TGATGCTGTA GAAACGTTAA TGTTTACGGA TTCGATCGAG 300

AATCTTCCTT TTCAGTATAG ACATTTTGTG ATTCCTTCGG GGACAGTGAT TAGGATGTTT 360

GGGAGATCTG AGGACGGTGA GAAGATTTGT GTGAACGTGT TTGGACAGGA GCAATATTTT 420

TACTGCGAAT GTGTCGACGG AAAAAGCCTG AAGGCTACTA TAAACAATTT GATGTTAACC 480

GGCGAGGTTA AGATGTCGTG TTCTTTTGTC ATTGAGCCGG CTGATAAGTT GTCATTGTAT 540

GGGTACAATG CCAACACTGT TGTTAATCTG TTTAAAGTGA GTTTTGGAAA TTTTTATGTA 600

TCTCAACGTA TTGGAAAGAT TCTGCAGAAT GAGGGATTCG TAGTTTATGA AATCGATGTA 660

GATGTTTTGA CTCGTTTCTT CGTCGATAAT GGTTTTTTGA GTTTCGGATG GTATAATGTA 720

AAAAAATATA TTCCTCAAGA TATGGGAAAA GGGAGTAATC TTGAGGTGGA AATTAACTGT 780

CATGTCTCGG ATTTAGTTTC TTTGGAAAAC GTCAATTGGC CCTTATATGG ATGCTGGTCC 840

TTCGACATAG AGTGTTTGGG CCAAAATGGG AATTTCCCGG ATGCCGAAAA TTTGGGTGAT 900

ATAGTTATTC AGATTTCTGT AGTTAGTTTT GATACTGAGG GCGACCGTGA TGAGAGACAT 960

CTGTTTACTC TGGGAACCTG TGAACAAATT GACGGCGTGC ATATATATGA ATTTGCGTCA 1020

GAGTTTGAAT TACTTTTGGG ATTTTTTATA TTTTTAAGGA TTGAGTCTCC GGAGTTTATT 1080

ACCGGTTATA ATATTAACAA TTTTGATTTA AAATATTTGT GTATAAGGAT GGATAAGATT 1140

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CCTCATGAAC AGTACAAGAA GGGGTTCCTT CAGGCGCAAA CCAAGGTGTT TACTTCCGGA 1260

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CTGGATACTA TTGCTAAGAT CTGTCTCCAG CAAGAAAAAG AGCAGTTATC GTACAAGGAA 1380

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CAGGACTCTG TCTTAGTTGT GCGTCTCTTT AAACAGATTA ATTATCATTA TGAGGTTGCC 1500

GAGGTCGCCA GATTGGCACA CGTCACGGCT AGATGTGTGG TGTTCGAGGG TCAGCAGAAG 1560

AAGATATTTC CCTGCATTCT TACGGAAGCA AAACGTCGTA ACATGATTCT TCCGAGTATG 1620

GTGTCTTCGC ATAATAGACA AGGGATAGGT TACAAAGGGG CTACCGTCTT GGAGCCTAAG 1680

ACGGGTTATT ATGCTGTGCC CACTGTGGTG TTTGATTTTC AGAGTTTGTA TCCGAGCATT 1740

ATGATGGCGC ATAATCTGTG TTATAGTACC TTAGTTTTGG ATGAACGGCA AATAGCTGGA 1800

TTATCAGAGA GTGACATCTT AACCGTGAAG TTGGGGGATG AGACCCATCG GTTTGTGAAG 1860

CCTTGTGTTC GCGAGTCTGT GCTTGGAAGT CTACTAAAGG ACTGGCTGGC CAAGAGACGA 1920

GAAGTGAAGG CGGAGATGCA AAACTGTTCG GATCCAATGA TGAAACTCCT TCTGGATAAA 1980

AAGCAGCTCG CTCTCAAAAC AACATGTAAC TCGGTGTACG GTGTCACGGG AGCGGCGCAC 2040

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TGTTCCACGG TGGATTATGT TAATTCCAAG ATGCAGTCCG AGCAATTCTT TTGCGAAGAA 2160

TTGGGTCTAA CGGCATCAGA TTTTACTGGT GATTTAAAAG TGGAGGTAAT CTATGGTGAT 2220

ACGGATAGCA TCTTTATGTC TGTCAGAAAT ATGGCTAATG AGTCTCTGCG GAGGATTGCG 2280

CCGATGATCG CCAAACATAT CACAGATCGC TTGTTCAAAT CGCCTATCAA GCTCGAGTTC 2340

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TTGGATGAAA CGTTTTCTGC TATTGCAGAG GTAATGTGA 3039

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2493 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..2493
(D) OTHER INFORMATION: /standard_name= "Glycoprotein gene sequence from MS1206 virus"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

ATGAGCAAGA TGAGAGTATT ATTCCTGGCT GTCTTTTTGA TGAATAGTGT TTTAATGATA 60

TATTGCGATT CGGATGATTA TATCAGAGCG GGCTATAATC ACAAATATCC TTTTCGGATT 120

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ACCTACACTT TTCCAGTGAG AACATATAAA AACGAGCTGA CGTTCCAAAC CAGTTACCGT 300

GATGTGGGTG TGGTTTATTT TCTGGATCGG ACGGTGATGG GTTTGGCCAT GCCGGTGTAC 360

GAAGCAAATT TAGTTAATTC TCGTGCGCAG TGTTATTCAG CCGTAGCGAT AAAACGACCC 420

GATGGTACGG TGTTTAGTGC CTATCATGAG GATAATAATA AAAACGAAAC TCTAGAATTA 480

TTTCCTCTGA ATTTCAAGTC TGTTACTAAT AAAAGATTTA TCACTACGAA AGAACCCTAC 540

TTTGCAAGGG GTCCTTTGTG GCTCTATTCT ACATCGACGT CTCTCAATTG TATTGTGACG 600

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GTGGAAGGGT CTCCGTTCTT CGACGGTTCA AACGGTAAAC ATTTTGCAGA GCCGTTAGAA 720

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TTCGTCGCCT CCAAGGAGTC TTTAAATCTT ACCGATCCCA AACAAACGTG TATTAAGAAT 1020

GAATTTGAGA AGATAATTAC AGATGTCTAT ATGTCAGATT ATAATGATAC ATACAGCATG 1080

AACGGTAGTT ATCAAATTTT TAAGACTACG GGAGATCTGA TTTTGATTTG GCAGCCTCTT 1140

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GCGAAGGGAG TAAGGAGTGA AACGATGTGT TATAATCGGC CCTTGGTGAC GTTTAGCTTT 1560

GTGAACTCCA CGCCTGAGGT TGTCCCTGGT CAGCTAGGGT TAGATAATGA GATTCTGTTG 1620

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CATGCACACG TGTATACCGA TTATACGCAT ACGAATTCGA CGCCCATAGA AGACATTGAG 1740

GTATTGGATG CTTTTATTAG ACTAAAGATC GACCCTCTCG AAAATGCTGA TTTTAAACTA 1800

CTTGATTTAT ATTCGCCGGA CGAATTGAGT AGAGCAAACG TTTTCGATTT AGAGAATATT 1860

CTTCGTGAAT ATAACTCATA TAAGAGCGCA CTATATACTA TAGAAGCTAA AATTGCTACT 1920

AATACGCCGT CGTATGTCAA TGGGATTAAT TCTTTTTTAC AAGGGCTTGG GGCTATAGGC 1980

ACTGGATTGG GCTCGGTTAT AAGTGTTACG GCAGGAGCAC TTGGGGATAT TGTGGGTGGA 2040

GTGGTGTCTT TTTTAAAAAA TCCATTCGGG GGTGGTCTCA TGTTGATTTT AGCGATAGTA 2100

GTTGTCGTTA TAATAATTGT GGTTTTCGTT AGACAAAAAC ATGTGCTTAG TAAGCCTATT 2160

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ACTGTCGTCA AGACGCCTAG TGTTAAAGAT GCTGACGGGG GCACATCTGT TGCGGTTTCG 2280

GAAAAAGAGG AGGGTATGGC TGACGTCAGT GGACAAATAA GTGGTGATGA ATATTCACAA 2340

GAAGATGCTT TAAAAATGCT CAAGGCCATA AAGTCTTTAG ACGAGTCCTA CAGAAGAAAA 2400

CCTTCGTCTT CTGAGTCTCA TGCCTCAAAA CCTAGTTTGA TAGACAGGAT CAGGTATAGA 2460

GGTTATAAGA GTGTAAATGT AGAAGAAGCG TGA 2493

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1692 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..1692
(D) OTHER INFORMATION: /standard_name= "Phosphotransferase gene from MS1206 virus"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

ATGGACAACG GTGTGGAGAC ACCTCAAGGT CAAAAAACTC AGCCGATAAA TTTGCCACCA 60
GACAGGAAAA GGTTGAGAAA ACATGACGGA CTTGGAAAAG GTGTTAAACG AAAACTTTTT 120
GCCGAAGATA GTTCTCCGTT AAAGAAACAG ATCCCCGCCT GCAGCGATAT GGAAACACTT 180
TCTTCGCCTG TAAAGTTTGG ATGCAAGTCG CGAAGTGCTT CTGCTCTCGA TGAAAGTTTC 240
GGAAAATGTA AACACGAAAC TGCTTGCGAT TGTTCTGCGA TAGAGGAATT GCTTTGTCAC 300
GAGTCGCTTT TAGACTCGCC GATGAAACTG TCGAATGCCC ACACCATGTT CAGCTCAGAC 360
AAATGGAAAC TGGAGCTAGA GAAAATTATA GCTTCAAAGC AGATATTTCT AGACATGAGC 420
GAGAATGTTG AACTTGTGGC CTACGGCGAG ACTTTGTGTA ACCTGAGAAT TTTCGAAAAG 480
ATCAGCTCGC CGTTTTTGTT TGACGTGCAA AGCGAGGAGC GTTCGTATTC AGTGGTTTAC 540
GTCCCTCACA ACAAAGAACT TTGTGGACAG TTTTGTCAAC CTGAAAAGAC TATGGCTCGA 600
GTTCTCGGAG TGGGTGCCTA CGGGAAGGTG TTTGATATAG ATAAAGTGGC CATAAAGACG 660
GCCAACGAAG ACGAGAGTGT CATTTCGGCT TTCATAGCCG GTGTCATCCG TGCAAAATCG 720
GGAGCCGACT TATTATCTCA CGACTGTGTT ATTAATAACC TTCTGATTTC AAATTCCGTT 780
TGTATGGATC ATAAAGTGTC TTTGTCACGT ACTTATGATG TTGATCTCTA TAAGTTCGAA 840
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GTAAGGTTTC TAAATCTGAA ATGTAGAATT AATCATTTCG ATATCTCACC TATGAATATC 960
TTTATAAATC ATAAAAAAGA GATAATCTTT GATGCCGTGT TGGCGGATTA CAGCTTGTCC 1020
GAAATACATC CCGAGTATAA CGGCACGTGT GCTATTGCTA AAGAGTATGA CAGAAATCTT 1080
CAACTTGTGC CAATCAGTCG TAACAAATTC TGTGACATGT TTAATCCTGG ATTTCGACCA 1140
CTTGTCGCCA ATGCAATGAT ATTGGTCAAT GTATGCGAGG CTTTTGATGG TGAAAATAAT 1200
CCTCTTAGAC ACTGTAATTT GGATCTGTGC GCCTTTGCTC AGGTCGTATT ATTGTGTGTC 1260
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TTTGCGTTGG CTAACGAGGC CTGTCGATTG AATCCTCTTA GATATCCATT TGCTTACAGG 1380
GATGCTTGCT GTAAAGTATT GGCTGAGCAT GTAGTGTTGC TAGGGTTATT GTTTTACCGA 1440
GACGTGGTTG ATATATATGA AAAAATATAC GATTTTCTAG ATGAAAGAGG GGAATTTGGG 1500
TTACGAGACC TGTTTGAGGC AACTTTTTTA AATAATAGTA AACTTACCAG ACGTCAGCCA 1560
ATCAGAGGAG GTCTTGCGTC TCTACAGTCG TCCGAGTATG GAGAAAAACT TTTACATGAC 1620
CTTAGAGCGT TGTTCTTGAT CACTTCTTCT GCAGATCTGG ATAAAGATAC ATCATCTCTC 1680

TTTCAGATGT GA 1692

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 445 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..445
(D) OTHER INFORMATION: /standard_name= "bases 2413-2858 from U1102"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

TTGGAGTGAC AGACAACGTC TGAATGGTAG GTAGCTCGTT TGAGGATTTC AAGGTTGTCC 60

TCTGGAATTT GTCGGTCATG TTTTGTACTT CTGTGTTGTG ACTCATGTAT GTGCGTACCG 120

TGTTTTGCCA AGTGGTAAAC TTTAAAATCG GAGTGGTGTG TTCGGCGTCG GTGAATTTTG 180

TTGTACTCTG CTGTGATTTG CTCTTAAGAT AAATAGAGTT TTTTGGTGAA GAATAGGTAT 240

TGCCGTTAAT TTCCTTGATT CCTATTGTTG TGAAGGTGGT GCTTTCGATC TTTTGGGTTG 300

CGTTTGTTGT TGCTGTAGCG CTCTCAAGGG TTTTGGGAAT TCTGATTGTG GTATTTTCAA 360

AATTTGTTGG TTTTGGTGGA CTTTTTGGGT TTTCGGGTTT TGTTCGGTTT TAATAGTGGC 420

GTTAAGACGG ATTGTAAGAG TTGGA 445

WHAT IS CLAIMED IS:

1. A method for treatment of multiple sclerosis (MS) comprising administering to a patient having virus associated MS a pharmaceutically effective amount of an antiviral agent in a pharmaceutically acceptable carrier wherein the agent is effective against human herpesvirus subtype 6 (HHV-6), with the proviso that the agent not be a β-interferon or cladribine unless said agents are in combination with other antiviral agents.

2. The method of claim 1 wherein the virus is HHV-6B2.

3. The method of claim 1 wherein the virus has the following DNA subsequence in its genome:
MS: TATTCCCTATTAGCCAAGCTTACAAATTTCTCTAATGTCAA Seq. ID. No. 17.

4. The method of claim 1, wherein the antiviral agent is a nucleoside analogue.

5. The method of claim 1, wherein the antiviral agent is selected from the group consisting of acyclovir, cygalovir, and ganciclovir.

6. The method of claims 1 wherein the antiviral agent is Poly(I)·Poly(C_{12}U).

7. The method of claim 1, wherein the antiviral agent is an antisense nucleic acid specific for HHV-6.

8. The method of claim 1, wherein the antiviral agent is an immune globulin from persons having a detectable titer of antibodies binding to HHV-6.

9. The method of claim 1 wherein the agent is applied directly to cerebrospinal fluids.

10. The method of claim 1 wherein the agent is an antibody which binds to human herpesvirus subtype 6.

11. The method of claim 1 wherein the agent are viral DNA polymerase inhibitors.

12. The method of 11 wherein the agents are selected from the group consisting of idoxuridine, trifluridine, vidarabine, acyclovir, brivudine, foscarnet and GS504 (HMPG).

13. The method of 1 wherein the agents are selected from the group consisting of brovavir, pencyclovir, famcicyclovir, desciclovir, bishydroxymethylcyclobutylguanine, and 1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine.

14. A method for the treatment of multiple sclerosis due to an infection of HHV-6 comprising adminstering directly to a patient's cerebrospinal fluid an anti-viral agent in an amount effective to treat the disease.

15. The method of claim 14 wherein the agent is applied through an intrathecal shunt.

16. The method of claim 14 wherein the agents are viral DNA polymerase inhibitors.

17. A medicament for the treatment of viral associated multiple sclerosis comprising an anti-herpesvirus agent and a pharmaceutical excipient in an amount effective to prevent or alleviate the symptoms of multiple sclerosis.

18. An isolated virus of the *Herpesviridae* family wherein the virus has the following DNA subsequence in its genome:

MS: TATTCCCTATTAGCCAAGCTTACAAATTTCTCTAATGTCAA Seq. ID. No. 17.

19. A composition of viral biologics selected from the group of proteins consisting of:

the HHV-6 DNA polymerase encoded by the nucleic acid sequence ID. No. 82; and,

the HHV-6 phosphotransferase encoded by the nucleic acid sequence ID. No. 84.

20. An isolated nucleic acid encoding either the HHV-6 DNA polymerase encoded by the nucleic acid sequence ID. No. 82; or the HHV-6 phosphotransferase encoded by the nucleic acid sequence ID. No. 84.

21. A method of screening for pharmaceutical agents useful in treatment of multiple sclerosis (MS) comprising:

contacting a cell, infected with HHV-6 that is associated with multiple sclerosis, with an anti-viral agent; and,

assaying the anti-viral agent activity by determining the effect of the agent upon viral titer in the cell.

22. The method of claim 21 where the cells are neuronal or glial cells.

23. An in vitro method for screening for pharmaceutical agents useful in treatment of multiple sclerosis (MS) comprising:

(a) contacting at least one of the following enzymes:

i. the HHV-6 DNA polymerase encoded by the nucleic acid sequence ID. No. 82 or,

ii. the HHV-6 phosphotransferase encoded by the nucleic acid sequence ID. No. 84

with an antiviral agent suspected of inhibiting the enzyme and

(b) measuring the inhibitory effect of the antiviral agent.

24. A method of claim 23 wherein the enzyme is isolated and is free from infectious virus.

25. The method of claim 21, wherein the virus is has the following DNA subsequence in its genome:

MS: TATTCCCTATTAGCCAAGCTTACAAATTTCTCTAATGTCAA Seq. ID. No. 17.

26. The method of claim 21, wherein the virus is MSV-1206.

27. The method of claim 23, wherein the agent is screened in *in vitro* assays against cell lines infected with the virus, against cells producing an enzyme from a virus or against a purified viral enzyme.

28. The method of claim 21 wherein the agent is screened in *in vivo* assays where the virus is hosted by a mammal.

29. A method for the diagnosis, progression or prognosis of multiple sclerosis (MS) in a patient comprising:

detecting for the presence or absence of a virus from the HHV-6 in a sample from a patient to be tested for MS.

30. The method of claim 29 wherein the detecting uses Herpesviridae specific nucleic acid to duplex with the viral genome and detecting duplex formation.

31. The method of claim 29 wherein the detecting uses a *Herpesviridae* specific antibody to bind to viral protein and detecting binding of the antibody to the viral protein.

32. The method of claim 29 wherein the detection occurs at multiple time points.

33. A method of claim 29 wherein the virus has the following DNA subsequence in its genome:

MS: TATTCCCTATTAGCCAAGCTTACAAATTTCTCTAATGTCAA Seq. ID. No. 17.

34. The method of claim 29, wherein the presence or absence of neurotropic HHV-6 is detected.

35. The method of claim 29, wherein the presence or absence of the virus is detected by hybridization of a nucleic acid probe to a viral genomic DNA comprising Sequence ID No. 17.

36. The method of claim 29, wherein the presence or absence of the virus is detected by amplification of a viral genomic nucleic acid comprising Sequence ID No. 17.

37. The method of claim 29, wherein the patient sample is tissue from the brain or cerebrospinal fluid of the patient.

38. The method of claim 29, wherein at least one other diagnostic factor for MS is detected for in the patient.

39. A method of prophylaxis for multiple sclerosis (MS) comprising administering to a patient at risk for MS, a vaccine against MS comprising inactivated viral particles or peptides of a virus in a pharmaceutically acceptable carrier wherein the vaccine is effective against HHV-6 wherein the virus has the following DNA subsequence in its genome:
MS: TATTCCCTATTAGCCAAGCTTACAAATTTCTCTAATGTCAA Seq. ID. No. 17.

40. The method of claim 39, wherein the vaccine comprises peptides encoded by a nucleic acid sequence from MSV-1206 in a pharmaceutically acceptable carrier.

41. A method of prophylaxis or treatment for multiple sclerosis (MS) by administering to a patient at risk for MS, an antibody that binds to HHV-6 in a pharmaceutically acceptable carrier.

42. The method of claim 41, wherein the antibody is specific for MSV-1206 or specific for a peptide encoded by a nucleic acid sequence of MSV-1206, said antibody in a pharmaceutically acceptable carrier.

43. A substantially pure nucleic acid probe having a sequence which binds specifically to a subsequence of multiple sclerosis associated HHV-6 said subsequence consisting of Sequence ID Nos. 17.

44. A diagnostic kit for the diagnosis of multiple sclerosis (MS) comprising a container having a nucleic acid or an antibody specific for HHV-6 identified by its having the following DNA subsequence in its genome:

MS: TATTCCCTATTAGCCAAGCTTACAAATTTCTCTAATGTCAA Seq. ID. No. 17.

45. The kit of claim 44 wherein the virus is MSV-1206 and instructional material for carrying out a diagnostic test for MS.

46. A diagnostic kit for the diagnosis of multiple sclerosis (MS) comprising instructional material for detecting a herpes virus and a herpes virus specific binding agent.

47. The kit claim 46 wherein the binding agent is a nucleic acid.

48. The kit of claim 46 wherein the binding agent is an antibody.

49. The use of an anti-herpesvirus agent to produce a medicament for the treatment of multiple sclerosis.

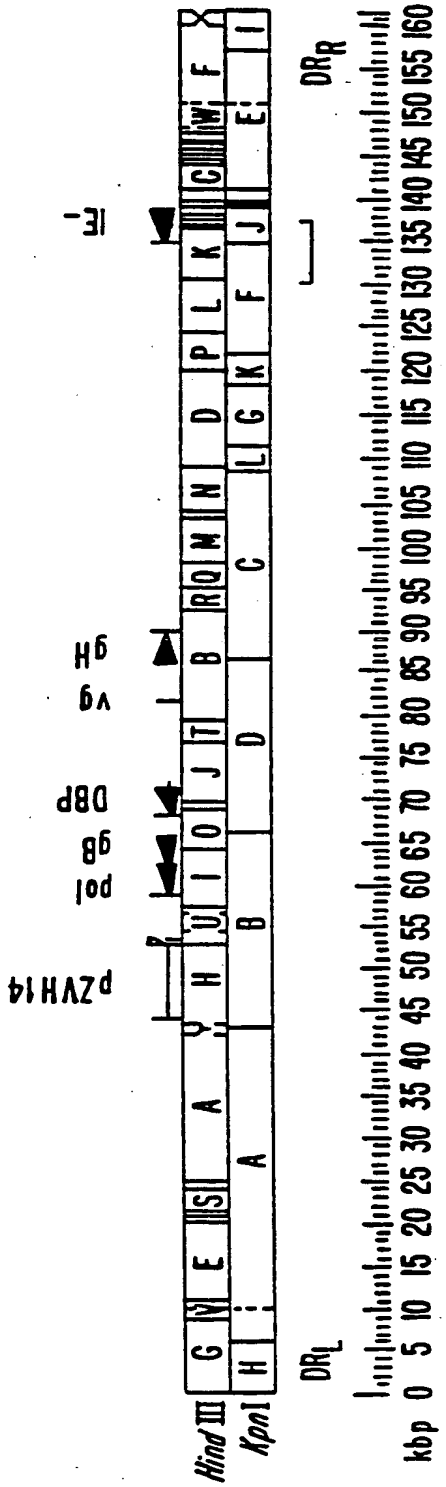
IE-
gH
vg
DBP
gB
pol
pZVH14
DR_R
DR_L
HindIII
KpnI
kbp 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160

FIG. 1.

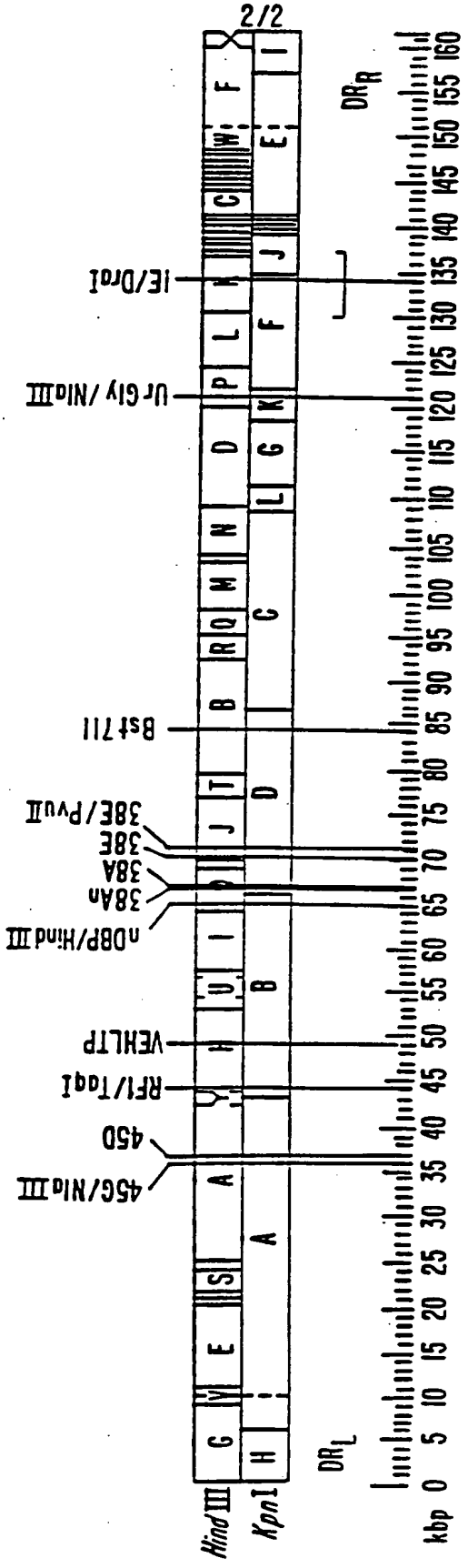
IE/DraI
Ur GIy /NlaIII
BstZ11
38E/PvuII
38E
38A
38An
nOBP/HindIII
VEHLTP
RF1/TaqI
45D
45G/NlaIII
HindIII
KpnI
DRL
DRR
kbp
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160

FIG. 2.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/12655

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 7.2, 183, 194, 235.1, 239; 514/2, 44; 530/350, 387.1; 536/23.1, 23.2, 23.5, 24.3, 24.31, 24.32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF NEUROLOGICAL SCIENCES, Volume 113, issued 1992, J.A.R. Nicoll et al., "PCR-mediated search for herpes simplex virus DNA in sections of brain from patients with multiple sclerosis and other neurological disorders", pages 144-151, see entire document.	1-6, 9, 11-20, 29-38, 42-47, and 49
Y	DEVELOPMENTAL BIOLOGY STANDARDS, Volume 70, issued 1989, D.V. Ablashi et al., "Utilization of human hematopoietic cell lines for the propagation and characterization of HBLV (human herpesvirus 6)", pages 139-146, see entire document.	1-6, 9, 11-20, 29-38, 42-47, and 49

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 31 JANUARY 1995	Date of mailing of the international search report 17 FEB 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BRIAN R. STANTON Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/12655

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	JOURNAL OF GENERAL VIROLOGY, Volume 74, issued 1993, U.A. Gompels et al., "Two groups of human herpesvirus 6 identified by sequence analyses of laboratory strains and variants from Hodgkin's lymphoma and bone marrow transplant patients", pages 613-622, see entire document.	18, 20, and 44-47 ----- 1-6, 9, 11-17, 19, 29-38, 42, 43, and 49
X -- Y	JOURNAL OF VIROLOGY, Volume 67, Number 8, issued August 1993, B. Pfeiffer et al., "Identification and mapping of the gene encoding the glycoprotein complex gp82-gp105 of human herpesvirus 6 and mapping of the neutralizing epitope recognized by monoclonal antibodies", pages 4611-4620, see entire document.	17, 18, 20, 44-46, and 49 ----- 1-6, 9, 11-16, 19, 29-38, 42, 43, and 47
Y	VIROLOGY, Volume 197, issued 1993, M.D. Jones et al., "Identification and Analysis of the transport/capsid assembly protein (tp/cap) gene of human herpesvirus-6 (HHV6)", pages 449-454, see entire document.	1-6, 9, 11-20, 29-38, 42-47 and 49
X -- Y	VIROLOGY, Volume 173, issued 1989, M.V. Williams et al., "Demonstration of the human herpesvirus 6-induced DNA polymerase and DNase", pages 223-230, see entire document.	19, and 44-46 ----- 1-6, 9, 11-18, 20, 29-38, 42, 47, and 49
Y	JOURNAL OF GENERAL VIROLOGY, Volume 74, issued 1993, D.X. Liu et al., "Identification and expression of the human herpesvirus 6 glycoprotein H and interaction with an accessory 40K glycoprotein", pages 1847-1857, see entire document.	1-6, 9, 11-20, 29-38, 42-47, and 49
X -- Y	JOURNAL OF NEUROLOGY NEUROSURGERY AND PSYCHIATRY, Volume 56, Number 8, issued 1993, P. Sola et al., "Human herpesvirus 6 and multiple sclerosis: Survey of anti-HHV-6 antibodies by immuno fluorescence analysis and of viral sequences by polymerase chain reaction", pages 917-919, see entire document.	17, 29, 31-34, 37, 42, 44-46, and 49 ----- 1-6, 9, 11-16, 18-20, 30, 35, 36, 38, 43, and 47
Y	VIROLOGY, Volume 175, issued 1990, D. DiLuca et al., "The replication of viral and cellular DNA in human herpesvirus 6-infected cells", pages 199-210, see entire document.	1-6, 9, 11-20, 29-38, 42, and 49

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/12655

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MOLECULAR PHARMACOLOGY, Volume 37, issued 1990, J. Balzarini et al., "Role of the incorporation of (E)-5-(2-Iodovinyl)-2'-deoxyuridine and its carbocyclic analogue into DNA of herpes simplex virus type 1-infected cells in the antiviral effects of these compounds", pages 402-407, see entire document.	1-6, 9, 11-18, and 49
Y	ANNALS OF INTERNAL MEDICINE, Volume 116, Number 2, issued 15 January 1992, D. Buchwald et al, "A chronic illness characterized by fatigue, neurologic and immunologic disorders, and active human herpesvirus type 6 infection", pages 103-113, see entire document.	1-6, 9, 11-20, 29-38, 42-47, and 49
X -- Y	GENBANK ENTRY HH6KAHC, issued 01 October 1993, T.R. Dambaugh et al., "A 20.9 kb domain of human herpesvirus 6B (Z29) DNA colinear with human cytomegalovirus encodes homologs of genes from discontinuous loci in alpha- and gammaherpesviruses and contains a putative origin of HHV-6B DNA replication", pages 37-42, see entire printout.	20, and 43-47 ----- 1-6, 9, 11-19, 29-38, 42 and 49

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/12655

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A01N 43/04; C07H 17/00; C07K 1/00, 14/03, 17/00; C12N 7/00, 7/01, 7/02, 9/00, 15/00, 15/09, 15/12; C12Q 1/00, 1/02, 1/68; G01N 33/53

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6, 7.1, 7.2, 183, 194, 235.1, 239; 514/2, 44; 530/350, 387.1; 536/23.1, 23.2, 23.5, 24.3, 24.31, 24.32

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

Databases: APS, Medline, CA, Biosis, Genbank, Embase
Search Terms: multipl?; sclero?; ms; cerobr?; review; hhv; herpes; simplex; vir?; 6; treat; phosphotrans?; burmer?/au; challoner?/au; smith?/au; brown?/au; parker?/au; nowinski?/au; msv?; interfer?; cladribin?; acyclovir; gancyclovir; ganciclovir; idoxuridine; vidarabine; brivudine; foscanet; phosphoracetic; gs504; pencyclovir; famcicyclovir; desciclovir; DNA; polymerase

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-5, 9, 11-17, 42, 43, and 49, drawn to methods of treating virus associated multiple sclerosis employing DNA synthesis inhibitors.
Group II, claims 1-4, 6, 9, 14, 15, 17, 42, and 49, drawn to methods of treating virus associated multiple sclerosis employing Poly I/C12U.
Group III, claims 1-4, 7, 9, 14, 15, 17, 42 and 49, drawn to methods of treating virus associated multiple sclerosis employing anti-sense nucleic acids.
Group IV, claims 1-4, 8-10, 14, 15, 17, 41, 42, 44-46, 48 and 49, drawn to methods of treating virus associated multiple sclerosis employing antibody compositions.
Group V, claims 1-4, 9, 14, 15, 17, 39, 40, and 42, drawn to methods of treating virus associated multiple sclerosis employing viral products.
Group VI, claims 18-20, 43 and 44 (in so far as claim 44 reads on HHV DNA), drawn to HHV virus particles, DNA therein and proteins encoded by said DNA.
Group VII, claims 21, 22, and 25-28, drawn to methods of screening pharmaceutical agents using cell based assay systems.
Group VIII, claims 23, 24 and 27, drawn to methods of screening pharmaceutical agents using protein based assay systems.
Group IX, claims 29-38, and 44-47, drawn to diagnostic methods.

The inventions listed as Groups I-IX do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I-V are distinct, one from the other because they are drawn to distinct methods of treating virus associated multiple sclerosis wherein disparate pharmaceutical agents are employed. For example, the methods of group I utilize DNA synthesis inhibitors that are based upon nucleoside analogues; the methods of group II utilize non-specific nucleic acids; the methods of group III utilize anti-sense nucleic acids; the methods of group IV utilize antibody compositions; and the methods of group V utilize viral gene products. Each of these pharmaceutical compositions are distinct chemical entities with divergent chemical and biological properties. Thus, the claimed inventions evidence that the claimed methods may be practiced in materially different fashions using disparate reagents and are therefore not so linked by any special technical feature so as to form a single inventive concept. The methods of any of groups I-V and any of the inventions of groups VI-IX are distinct, one from the other because the former groups of inventions are all drawn to in vivo treatment methods that involve alteration or modulation of disease etiology and none of the latter groups of inventions involve such in vivo activities.

The compositions of group VI and the methods of any of the inventions of groups VII-IX are distinct, each from the

other because the viral compositions may be used in materially different manners than those of the methods of groups VII-IX. For example, the viral compositions of group VI may be used in treatment methods (see e.g. the methods of group V).

The screening methods of the inventions of groups VII and VIII are distinct, one from the other because they utilize materially different processes to assay for therapeutic agents. In the case of the methods of group VII, cellular based assays are employed which involve consideration of the properties of cellular compositions and the biological effect of putative agents on such compositions. In contrast, the methods of group VIII utilize purified proteins and in vitro assays which directly assess the effect of putative agents on defined enzymatic activities. Consequently, no consideration of complex cellular systems is required.

The methods of either of groups VII or VIII and the diagnostic methods of group IX are distinct, one from the other because the latter methods involve determination of disease states in an individual and therefore require consideration of such states. The former methods do not require consideration of any disease status of an individual, per se.
For the foregoing reasons, the above listed groups inventions are not so linked by any single inventive concept within the meaning PCT Rule 13.1 because they lack a unifying special technical feature.